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工學博士 學位論文

**Applications of *N*-hydroxymethyl protected
 α -amino aldehydes for stereoselective synthesis of
threo- β -amino- α -hydroxy acids and
Cu-catalyzed one-pot process for acetaminophen**

N-하이드록시 메틸기를 갖는 α -아미노 알데하이드를
활용한 *threo*- β -아미노- α -하이드록 카복실산의 입체선택적
합성과 아세트아미노펜의 구리 촉매화 단일단계 공정개발

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**Applications of *N*-hydroxymethyl protected
 α -amino aldehydes for stereoselective synthesis
of *threo*- β -amino- α -hydroxy acids and
Cu-catalyzed one-pot process for acetaminophen**

by

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Abstract

This thesis comprises part A and part B. Part A is about the stereoselective synthesis of *threo*- β -amino- α -hydroxy acids from the *N*-hydroxymethyl protected α -amino aldehydes. *threo*- β -Amino- α -hydroxy acids, frequently found in naturally occurring bioactive compounds, have been synthesized via the stereoselective intramolecular conjugate addition of *N*-hydroxymethyl group, which configurationally stabilizes an α -amino aldehyde by forming the hemiacetal. In this study, the stable α -amino aldehyde was reacted with phenylsulfonylnitromethane ($\text{PhSO}_2\text{CH}_2\text{NO}_2$) to afford stereoselective *trans*-oxazolidine, which was a precursor for *threo*- β -amino- α -hydroxy acid. The three tandem reactions between phenylsulfonylnitromethane and the α -amino aldehydes and the followed in-situ ozonolysis afforded *trans*-oxazolidine methyl esters (**A23**) in 65% to 69% yields with more than 20 to 1 stereoselectivity. Bestatin, a well-known aminopeptidase inhibitor, was stereoselectively synthesized in 83% yield from *trans*-oxazolidine methyl ester **A23-Phe**, which was derivated from D-Phe-OH. Leucinal and valinal derivatives (**A23-Leu**, and **A23-Val**) were successfully applied for the synthesis of the corresponding *threo*-vicinal amino alcohol embedded dipeptides at N-terminus, which were the first synthesized isobutyl and isopropyl substituted bestatin analogs. Moreover, two stereoselective dipeptides, containing (2*S*,3*R*), or (2*R*,3*S*)-3-amino-2-hydroxy-4-methylpentanoic acid at N-terminus, were synthesized to elucidate the stereochemistry of naturally occurring another aminopeptidase inhibitor lapstatin from the commercially available Boc-D-Val-OH or Boc-L-Val-OH.

trans-Oxazolidine methyl ester from D-Ser-OH (**A23-Ser**) was stereoselectively converted to the glutamate transporter blocker *L-threo*- β -benzyloxy aspartic acid (L-TBOA) via the simple functionalization of hydroxymethyl of the oxazolidine **A23-Ser** and following selective benzyl protection of hydroxyl group.

In part B, a new process development for acetaminophen has been presented. In the new process, two copper catalyzed reactions were applied with *p*-dihalobenzenes for practical and efficient synthesis of acetaminophen. *N*-Acetylamidomethyl and hydroxyl group on acetaminophen were regioselectively introduced via Goldberg reaction with acetamide and Ullmann type condensation reactions in the presence of copper catalysts and diamine ligands. Furthermore, the suggested efficient two-step process was successfully simplified as one-pot process by excluding the purification of the intermediate, *N*-acetyl-*p*-haloaniline. Under the optimized one-pot process, acetaminophen was obtained in 74% yield from the commercially available *p*-bromiodobenzene.

Keywords: *threo*- β -amino- α -hydroxy acids, stereoselective intramolecular conjugate addition, aminopeptidase inhibitors, *L-threo*- β -benzyloxy aspartic acid, acetaminophen, copper-catalyzed amidation, copper-catalyzed hydroxylation.

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Contents

Abstract	i
List of Figures	vi
List of Schemes	viii
List of Table.....	ix
List of Abbreviations.....	xi

Part A

Applications of *N*-hydroxymethyl protected α -amino aldehydes for stereoselective synthesis of *threo*- β -amino- α -hydroxy acids.. 1

Introduction 1

1. Introduction of stable α -amino aldehydes..... 1

2. The application of *N*-hydroxymethyl group in the stereoselective syntheses of γ -amino- β -hydroxy acids 3

3. The application of *N*-hydroxymethyl group in the stereoselective syntheses of β -amino- α -hydroxy acids..... 9

Results and Discussions 16

1. The stereoselective synthesis of *threo*- β -amino- α -hydroxy acids 16

2. Application of the stereoselective synthetic method for β -amino- α -hydroxy acids : bestatin and its derivatives 28

3. Application of the stereoselective synthetic method for β -amino- α -hydrox acids : *threo*- β -benzyloxyaspartate..... 36

4. Application of the stereoselective synthetic method for β -amino- α -hydrox acids : lapstatin and its derivatives 43

Conclusion 50

Experimental Details 51

Part B

Cu-Catalyzed one-pot process for acetaminophen.....	67
Introduction	67
1. Acetaminophen.....	67
2. Conventional process for acetaminophen	72
3. Regioselective halogenation for <i>p</i> -dihalobenzenes.....	79
4. Goldberg and Ullmann type condensation reactions.....	86
Results and Discussion	92
1. The development of a new efficient process for acetaminophen ..	92
2. Acetaminophen synthesis from <i>p</i> -diiodobenzene.....	94
3. Acetaminophen synthesis from <i>p</i> -dihalobenzenes	107
4. A New one pot process for acetaminophen	118
5. Acetaminophen production via the Cu-catalyzed process.....	120
Conclusion	122
Experimental Details	123
References	128
Appendices	142
1. List of ¹ H NMR Spectra of Selected Compounds.....	143
2. List of ¹³ C NMR Spectra of Selected Compounds.....	176
Abstract in Korean.....	209

List of Figures

Part A

Figure A 1. Reported α -amino aldehydes.....	1
Figure A 2. The synthesized γ -amino- β -hydroxy acids	8
Figure A 3. β -Amino- α -hydroxy acid units in natural products.....	9
Figure A 4. ^1H NMR of A22-Phe (40 °C, 600 MHz, CDCl_3)	19
Figure A 5. The spectrum of HSQC of A22-Phe	21
Figure A 6. The expanded spectrum of HSQC of A22-Phe	22
Figure A 7. The structure of bestatin	28
Figure A 8. The interaction between bestatin and the active site of APN	29
Figure A 9. The interaction of bestatin with hAPN.....	30
Figure A10. The interaction of amastatin with hAPN.....	31
Figure A11. The stereoselectively synthesized dipeptides	35
Figure A12. The conversion of L-Asp as the glutamate blocker	37
Figure A13. Aminopeptidase inhibitors from <i>Streptomyces</i>	43
Figure A14. Structure of lapstatin	44
Figure A15. The prepared dipepdes A35 and A36	46
Figure A16. The prepared dipepdes with <i>erythro</i> -AHMPA	49

Part B

Figure B 1. The structure of acetaminophen	67
Figure B 2. Structure of acetanilide and phenacetin.....	68
Figure B 3. Total sales of acetaminophen containing products in the US.....	70
Figure B 4. Total sales of analgesics & anti-inflammatroy drugs in Korea ..	71

Figure B 5. Possible colored impurities on the synthesis of <i>p</i> -aminophenol.....	76
Figure B 6. Continuous flow process for another analgesic, ibuprofen	77
Figure B 7. Common halogen-based oxidants	79
Figure B 8. Reported halogenation reaction conditions	80
Figure B 9. Reported methods for <i>p</i> -dihalobenzenes	83
Figure B10. Practical process for <i>p</i> -diiodobenzene.....	84
Figure B11. Various ligands for copper catalyzed amination.....	87
Figure B12. General substrates and ligands for arylamide synthesis.....	88
Figure B13. General substrates and ligands for ether formation.....	90
Figure B14. A new approach for efficient acetaminophen synthesis	91
Figure B15. Recovery of I ₂ in the phenylenediamine production	121

List of Schemes

Part A

Scheme A 1. The preparation of a stable α -amino aldehyde	2
Scheme A 2. Retroscheme for γ -amino- β -hydroxy acids.	3
Scheme A 3. Synthesis of (-)-statin with the <i>N</i> -hydroxymethyl group.....	4
Scheme A 4. Synthesis of (-)-deoxyaminostatin with <i>N</i> -amidomethyl group ...	5
Scheme A 5. Synthesis of <i>threo</i> - β -hydroxy-L-glutamic acid	6
Scheme A 6. Synthesis of <i>threo</i> - <i>N</i> -Boc-AHPPA	7
Scheme A 7. Synthesis of <i>erythro</i> - <i>N</i> -Boc-AHPPA.....	8
Scheme A 8. Sharpless asymmetric aminohydroxylation.	10
Scheme A 9. Opening of a chiral epoxide	10
Scheme A10. Asymmetric Mannich reaction.....	11
Scheme A11. Synthetic strategy for β -amino- α -hydroxy acids	12
Scheme A12. The Henry reaction with the stable α -amino aldehyde	12
Scheme A13. β -Amino- α -hydroxy acids synthesis with $\text{PhSO}_2\text{CH}_2\text{NO}_2$	14
Scheme A14. The absolute stereochemistry of A23-Phe	15
Scheme A15. Stereoselective synthesis of <i>threo</i> -AHPBA	17
Scheme A16 Proposed mechanism of oxidative Nef reaction with A22	18
Scheme A17. Proposed mechanism for the intramolecular conjugate addition	23
Scheme A18. Proposed transition state for high stereoselectivity.	25
Scheme A19. β -Amino- α -hydroxy acids from the one-pot process	25
Scheme A20. Retrosynthesis of bestatin	32
Scheme A21. Preparation of the starting material for bestatin synthesis	33
Scheme A22. Synthesis of bestatin	33

Scheme A23. Synthesis of betatin analogs, A29 and A30	34
Scheme A24. The previous synthetic route for L-TBOA	38
Scheme A25. Retrosynthetic route for L-TBOA	38
Scheme A26. The preparation of the stable serinal derivative	39
Scheme A27. The stereoselective synthesis of L-TBOA.....	40
Scheme A28. The stereochemistry of <i>ent</i> - A32-Ser	41
Scheme A29. Retrosynthesis of lapstatin from D-Valine	45
Scheme A30. Synthesis of lapstatin from Boc-D-Val-OH.....	46
Scheme A31. Stereodivergent synthesis of β -amino- α -hydroxy acids	47
Scheme A32. Model study on carbocyclization	47
Scheme A33. Ruthenium catalyzed oxidation with A23-Val and A24-Leu ...	48
Scheme A34. Epimerization of A23-Val for <i>erythro</i> -AHMPA synthesis.....	48

Part B

Scheme B1. Prostaglandin H ₂ production with COX.....	69
Scheme B2. Conventional process for acetaminophen.....	72
Scheme B3. Alternative processes for acetaminophen.....	74
Scheme B4. Goldberg and Ullmann condensation reactions	86
Scheme B5. Possible routes with <i>p</i> -diiodobenzene.....	92
Scheme B6. Acetamidation with PDIB under the optimized conditions.....	101
Scheme B7. The preparation of <i>N</i> -acetyl- <i>p</i> -iodoaniline.....	102
Scheme B8. The acetaminophen synthesis from <i>p</i> -diiodobenzene	107
Scheme B9. Acetaminophen production via Cu-catalyzed process	120

List of Table

Part B

Table B1. Catalysts screening on acetamidation	95
Table B2. Amino acid ligand screening results at amidation.	96
Table B3. Diamine ligand screening results at amidation	97
Table B4. Phosphate and carbonate bases screening at acetamidation.....	99
Table B5. Fluoride bases screening at acetamidation.....	100
Table B6. Fluoride bases screening at acetamidation.....	101
Table B7. Screening results of catalysts at hydroxylation of <i>N</i> -acetyl- <i>p</i> - iodoaniline.....	103
Table B8. Screening results of bases at hydroxylation of <i>N</i> -acetyl- <i>p</i> - iodoaniline.....	104
Table B9. Screening results of co-solvent at hydroxylation of <i>N</i> -acetyl- <i>p</i> - iodoaniline.....	105
Table B10. Results on normal heating system	106
Table B11. Optimization of the reaction conditions for acetamidation.....	110
Table B12. Acetamidation with various halobenzenes.....	113
Table B13. Optimization of the reaction conditions for hydroxylation.....	115
Table B14. CuI-catalyzed hydroxylation with various halobenzenes	116
Table B15. One-pot procedure for acetaminophen	119

List of Abbreviations

AcOH	Acetic acid
Ac₂O	Acetic anhydride
AHMH	3-Amino-2-hydroxy-5-methylhexanoic acid
AHMPA	3-Amino-2-hydroxy-5-methylpentanoic acid
AHPBA	3-Amino-2-hydroxy-4-phenylbutanoic acid
APN	Aminopeptidase N
Boc	<i>tert</i> -Butyloxycarbonyl
Bn	Benzyl
BNAH	<i>N</i> -Benzylnicotinamide
Cbz	Carboxybenzyl
CHDA	Cyclohexane-1,2-diamine
COX	Cyclooxygenase
<i>m</i>-CPBA	<i>m</i> -Chloroperoxybenzoic acid
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DIBAL	Diisobutylaluminium hydride
DMAP	4-(Dimethylamino)pyridine
DMCHDA	<i>trans</i> - <i>N,N'</i> -Dimethylethylenediamine
DMEDA	<i>N,N'</i> -Dimethylethylenediamine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethylsulfoxide
EA	Ethyl acetate (EtOAc)
EDA	Ethylenediamine
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
HOBt	Hydroxybenzotriazole
HSQC	Heteronuclear single quantum coherence spectroscopy
GC-MS	Gas chromatography Mass spectrometry

KHMDS	Potassium hexamethyldisilazide
LAH	Lithium aluminium hydride
LHMDS	Lithium hexamethyldisilazide
MsCl	Methanesulfonyl chloride
NMR	Nuclear magnetic resonance
PDIB	<i>p</i> -Diiodobenzene
PPTS	Pyridinium <i>p</i> -toluenesulfonate
TBAF	Tetrabutylammonium fluoride
TBHP	<i>tert</i> -Butyl hydroperoxide
TBOA	<i>threo</i> -β-Benzoyloxyaspartate
TBS	<i>tert</i> -Butyldimethylsilyl
TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy free radical
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TLC	Thin layer chromatography
TMEDA	<i>N,N,N',N'</i> -Tetramethylethylenediamine
TMS	Tetramethylsilane, Trimethylsilyl
TMSCHN₂	Trimethylsilyldiazomethane
TPAP	Tetrapropylammonium perruthenate
Ts	<i>p</i> -Toluenesulfonyl
<i>p</i>-TsOH	<i>p</i> -Toluenesulfonic acid

Chapter A

Applications of *N*-hydroxymethyl protected α -amino aldehydes for stereoselective synthesis of *threo*- β -amino- α -hydroxy acids

Introduction

1. Introduction of stable α -amino aldehydes

There had been a few configurationally stable α -amino aldehydes due to the chemically and configurationally labile proton at α -position on the α -amino aldehyde even though they were useful chiral synthons in asymmetric synthesis.¹ The reported stable α -amino aldehydes and their limitations were shown in Figure A1.

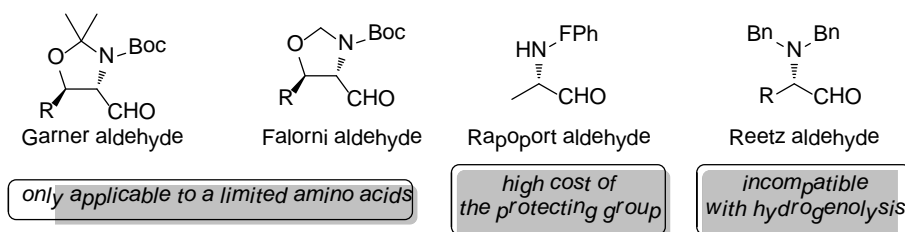
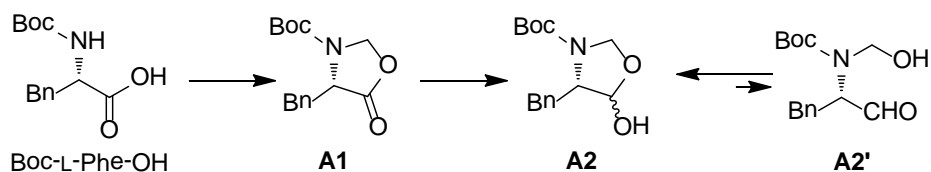


Figure A1. Reported α -amino aldehydes.

In order to overcome the limitations, a saturated hemiacetal group was suggested instead of the carbonyl group of *N*-protected α -amino aldehydes,

which was suggested by Ito *et al.*³ The conversion of carbonyl group to the saturated hemiacetal would be completed by the introduction of an *N*-hydroxyalkyl group to the aldehyde. With Boc protected phenylalanine, the stable α -amino aldehyde was prepared via the condensation with paraformaldehyde (**A1**) and the following reduction to the corresponding hemiacetal, lactol (**A2**, Scheme A1).⁴



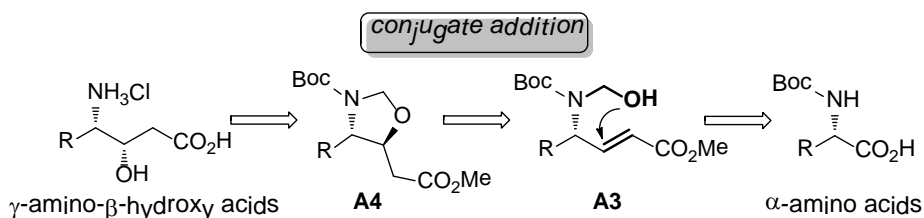
Scheme A1. The preparation of a stable α -amino aldehyde.

The stability of the prepared α -amino aldehyde **A2** was confirmed by the weathering test with diol compounds (not shown), which was obtained by the reduction of the lactol **A2** with LAH.⁴ Careful analysis of ^1H NMR spectra indicated 1-1.5% racemization during the preparation of lactol **A2**, and no racemization was observed during storage at room temperature. However, additional 1-1.5% racetamization was observed when the sample stored at room temperature for a month. The stabilizing effect from the *N*-hydroxymethyl group was quite evident compared with 3-5% racemization during the preparation of Garner aldehyde.^{2a}

2. The application of *N*-hydroxymethyl group

in the stereoselective syntheses of γ -amino- β -hydroxy acids

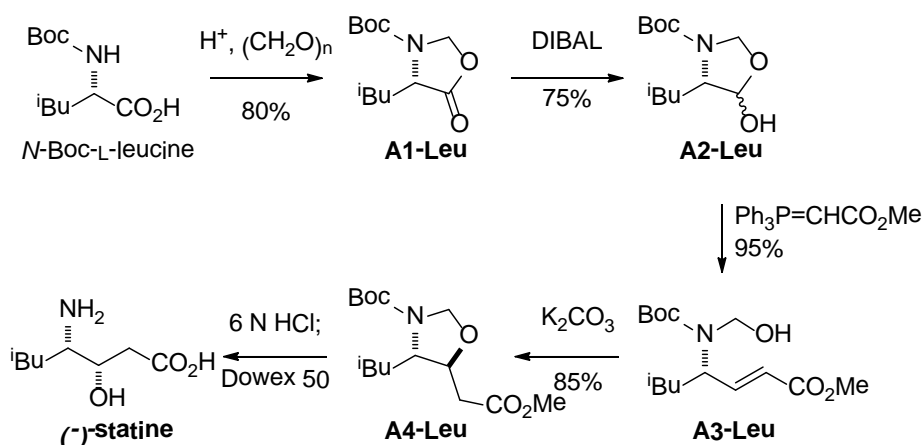
The *N*-hydroxymethyl group on the stable α -amino aldehyde (**A2**) did not only stabilize the labile α -amino aldehyde by forming lactol, but also was utilized as a crucial element for the intramolecular conjugate addition. *threo*- γ -Amino- β -hydroxy acids, found in a number of bioactive natural compounds like peptide enzyme inhibitors, amino sugar antibiotics, alkaloids and sympathomimetic amines,⁵ had been efficiently synthesized with the stable α -amino aldehydes. The intramolecular conjugate addition between the *N*-hydroxymethyl and the introduced internal electrophile α,β -unsaturated methyl ester afforded the stereoselective intermediate **A4** (Scheme A2).



Scheme A2. Retroscheme for γ -amino- β -hydroxy acids.

Contrast to the previously reported α -amino aldehydes, such as Garner aldehyde and Falorni aldehyde, the hemiacetal form of the stable α -amino aldehyde **A2** could be synthesized from various amino acids. From the practical advantage on the preparation of lactol **A2**, the synthetic method for γ -amino- β -hydroxy acids had been applied to synthesize (-)-statin, (-)-

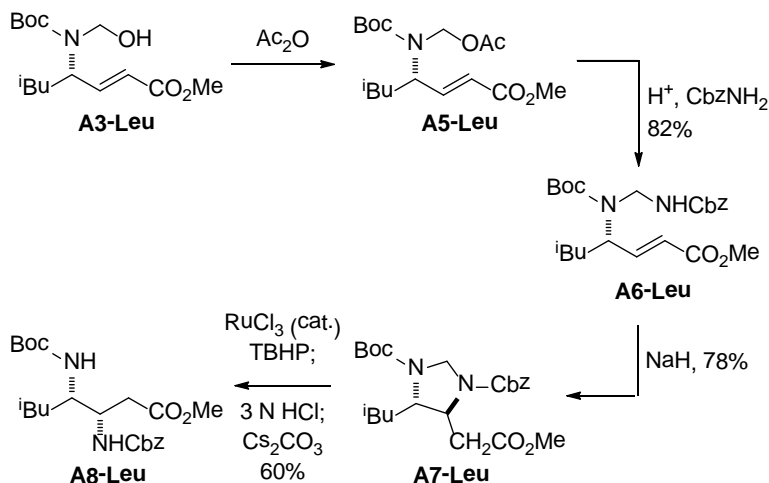
aminodeoxy statin, β -hydroxyglutamic acid and *threo*-AHPPA from the commercially available Boc-Leu-OH, Boc-Ser-OH and Boc-Phe-OH. Moreover, the developed synthetic method had been expended to synthesize *erythro*-AHPPA via the intramolecular epoxidation with *N*-hydroperoxymethyl group, which was prepared in-situ by the oxidation of the *N*-hydroxymethyl group.



Scheme A3. Synthesis of (-)-statin with the *N*-hydroxymethyl group.

The first application was started with the preparation of lactol from Boc-L-Leu-OH (Scheme A3).⁶ With the synthesized leucinal derivative **A2-Leu**, the introduction of the internal electrophile α,β -unsaturated methyl ester was completed via Wittig olefination with stabilized ylide. The prepared (*E*)- γ -amino- α,β -conjugate ester **A3-Leu** undergone the intramolecular conjugate addition of the *N*-hydroxymethyl group under the basic reaction condition. Therefore, the stereoselective intermediate *trans*-oxazolidine was synthesized with more than 10:1 stereoselectivity. The synthesized oxazolidine **A4-Leu**

was readily converted to enantiomerically pure (-)-statin from the simple hydrolysis and the following purification with ion exchange resin chromatography.

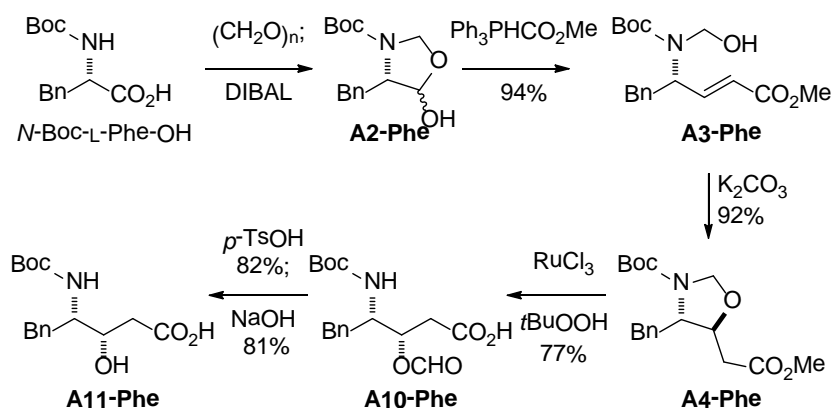


Scheme A4. Synthesis of (-)-deoxyaminostatin with *N*-amidomethyl group.

Based on the successful synthesis of enantiomerically pure (-)-statin, (-)-aminodeoxystatin,⁷ one of the derivatives, was synthesized via the same intramolecular conjugate addition after converting the *N*-hydroxymethyl group to *N*-aminomethyl group (Scheme A4). The synthesized aminodeoxystatin showed 10 to 1 stereoselectivity, like (-)-statin.

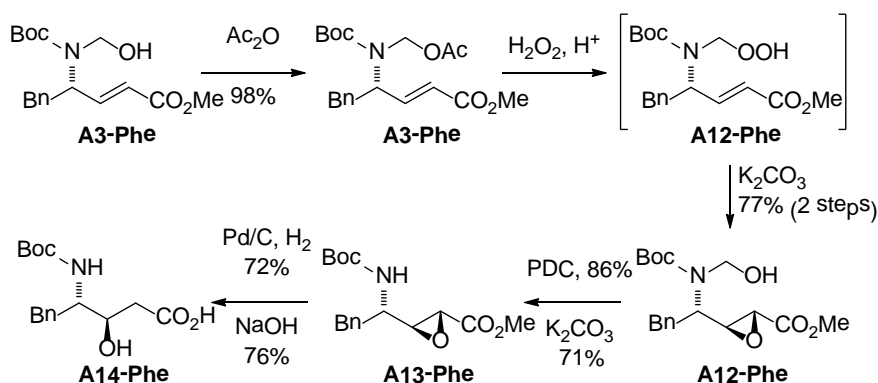
The next application of the stereoselective intramolecular conjugate addition of the *N*-hydroxymethyl group was the synthesis *threo*- β -hydroxy-L-glutamate (Scheme A5).⁸ A suitable amino acid for the target compound, Boc-L-Ser-OH, was readily converted to lactol (**A2-Ser**) via the same procedure for the preparation of **A2-Leu**. However, low stereoselectivity was obtained

from Boc-L-Phe-OH in three step (Scheme A6). The stereoselectivity of the intramolecular conjugate adduct **A4-Phe** was estimated at more than 20 to 1. The oxidative *N, O* acetal cleavage of the *trans*-oxazolidine methyl ester (**A4-Phe**) presented *O*-formylated **A10-Phe**, and the followed sequential acidic and basic hydrolysis afforded *N*-Boc protected *threo*-4-amino-3-hydroxy-5-phenylpentanoic acid, *threo*-*N*-Boc-AHPPA.



Scheme A6. Synthesis of *threo*-*N*-Boc-AHPPA.

In the synthesis of *erythro*-*N*-Boc-AHPPA, a new synthetic method with the *N*-hydroxymethyl group had been applied to the intramolecular epoxidation via the conversion of the *N*-hydroxymethyl group to the *N*-hydroperoxymethyl group.¹⁰ Based on the newly developed intramolecular epoxidation, *anti*-epoxide **A12-Phe** was prepared more than 20 to 1 ratio and converted to the *N*-Boc protected *erythro*-AHPPA via the removal of *N*-hydroxymethyl group and the followed Pd-catalyzed hydrogenation to give the corresponding amino alcohol **A14-Phe**.



Scheme A7. Synthesis of *erythro*-*N*-Boc-AHPPA.

The following figure showed all of the synthesized γ -amino- β -hydroxy acids via the stereoselective intramolecular conjugate addition (Fig. A2).

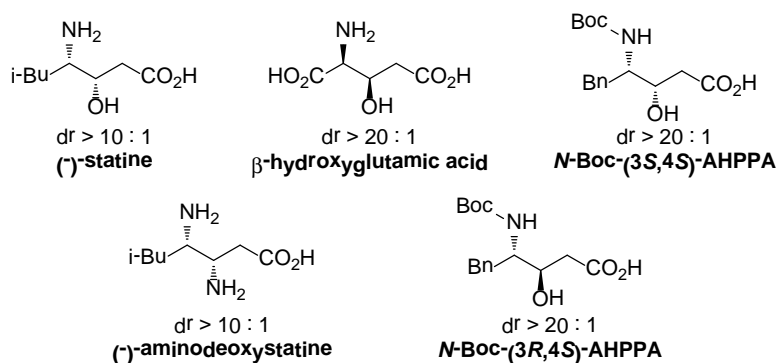


Figure A2. The synthesized γ -amino- β -hydroxy acids.

From the successful application of the *N*-hydroxymethyl group to the stereoselective synthesis of γ -amino- β -hydroxy acids,⁶⁻¹⁰ another series of vicinal amino hydroxy acids had been planned as the extension of the intramolecular conjugate addition of the *N*-hydroxymethyl group.

3. The application of *N*-hydroxymethyl group

to the stereoselective synthesis of β -amino- α -hydroxy acids

The development of an efficient synthetic method for β -amino- α -hydroxy acids had been an attractive subject since non-proteinogenic amino acids had been frequently found in biologically active natural products.¹¹ For example, typical β -amino- α -hydroxy acid units were embedded in an anticancer and antibacterial agent bestatin,¹² an aminopeptidase inhibitor amastatin,¹³ an glutamate transporter blocker *L-threo*- β -benzyloxyaspartic acid (L-TBOA),¹⁴ and an anticancer paclitaxel¹⁵ (Fig. A3). Some β -amino- α -hydroxy acids had been also used as chiral synthons or auxiliaries.¹⁶

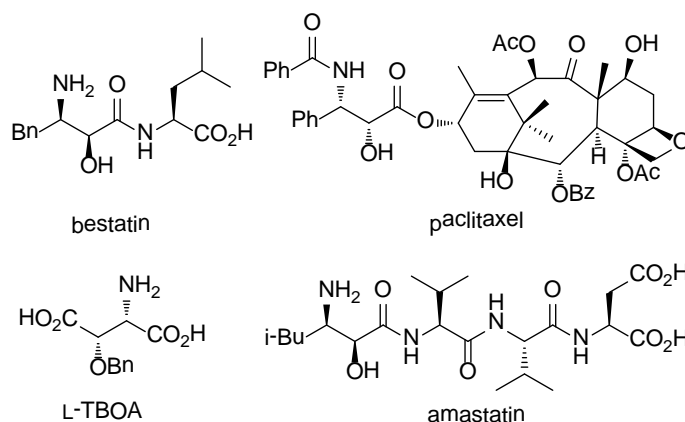
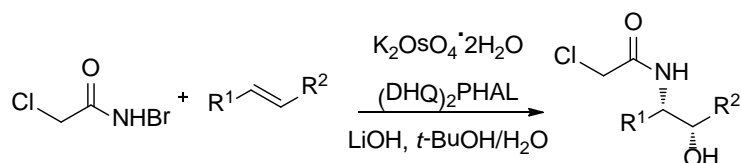


Figure A3. β -Amino- α -hydroxy acid units in natural products

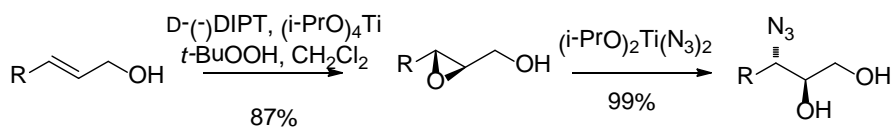
Enantiomerically pure β -amino- α -hydroxy acids had been synthesized mostly via reagent-controlled synthetic methods such as the asymmetric aminohydroxylation,¹⁷ the selective opening of a chiral epoxide,¹⁸ and the asymmetric Mannich reaction.¹⁹

The aminohydroxylation provided one-step stereospecific conversion to the protected β -amino alcohols using the catalytic amount of osmium source and chiral ligands based on cinchona alkaloids (Scheme A8). However, the asymmetric aminohydroxylation required chiral reagents and showed limitation of substrates.



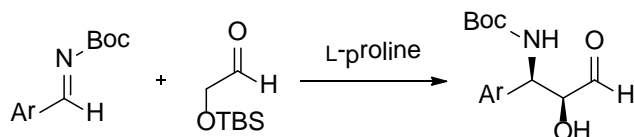
Scheme A8. Sharpless asymmetric aminohydroxylation.

The opening of a chiral epoxide was another method for high enantiomeric excess (Scheme A9). Although high enantiomeric excess and acceptable yields had been observed, this method required many reaction steps for the preparation of a chiral epoxide and the functionalization after the ring opening, and this method also showed limitation of substrates.



Scheme A9. Opening of a chiral epoxide.

In asymmetric Mannich reaction, β -amino- α -hydroxy aldehyde had been synthesized with high stereoselectivity. Asymmetric Mannich reaction was simple but showed low yields in the presence of chiral catalysts.

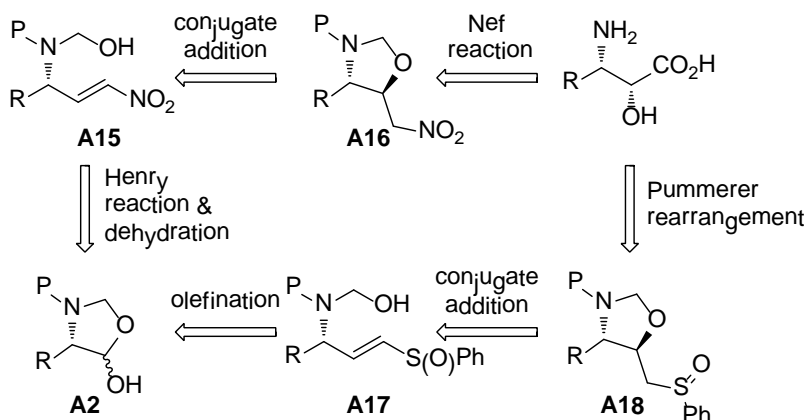


Scheme A10. Asymmetric Mannich reaction.

Based on the simplicity and diverse utility of the *N*-hydroxymethyl group on the stable α -amino aldehyde, the intramolecular conjugate addition of it had been applied to synthesize stereoselective β -amino- α -hydroxy acids. In the previous reports on the stereoselective synthesis of γ -amino- β -hydroxy acids, an α,β -unsaturated ester group was used as an efficient Michael acceptor of the *N*-hydroxymethyl group (Scheme A2).⁶⁻¹⁰ Contrast to the previous studies, a nitroolefin **A15**, or a conjugated arylsulfoxide **A17** was envisioned for the synthesis of one-carbon less Michael acceptor than γ -amino- β -hydroxy acids (Scheme A11). The nitromethyl group of **A16** or the arylsulfinyl group of **A18**, resulted from the intramolecular conjugate addition between the *N*-hydroxymethyl group and the introduced internal Michael acceptor, could be easily transformed into a carboxylic acid or an aldehyde via the Nef reaction,²⁰ or the Pummerer rearrangement,²¹ respectively.

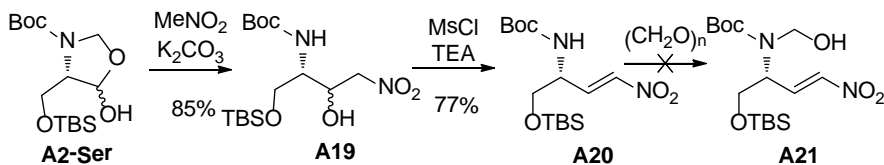
Configurationally stable α -amino aldehyde **A2** (R = Bn), a starting material in Scheme A11, was obtained in two steps from commercially available *N*-Boc-L-phenylalanine by following the reported procedure.⁴ In the beginning, the installation of the conjugated phenylsulfinyl group into **A2** was tried via the Horner-Wadsworth-Emmons olefination with (diethylphosphoryl)methyl-phenylsulfoxide,²² or via the Peterson olefination with trimethylsilylmethylphenyl-sulfoxide.²³ However, any of the desired

intermediates with a phenylsulfinyl group of **A17** or **A18** was not afforded.



Scheme A11. Synthetic strategy for β -amino- α -hydroxy acids.

Then, the introduction of another potent Michael acceptor, a nitroolefin group, was attempted in order to obtain the nitroolefin **A15** in Scheme A11. The Henry (nitro-aldol) reaction²⁴ of **A2-Ser** with nitromethane under conventional reaction conditions produced a nitro-aldol adduct without the *N*-hydroxymethyl group (**A19**, Scheme A11) whose instability under either basic or acidic conditions had been reported before.^{4a} The reinstallation of the *N*-hydroxymethyl was failed to afford the desired product under various reaction conditions.

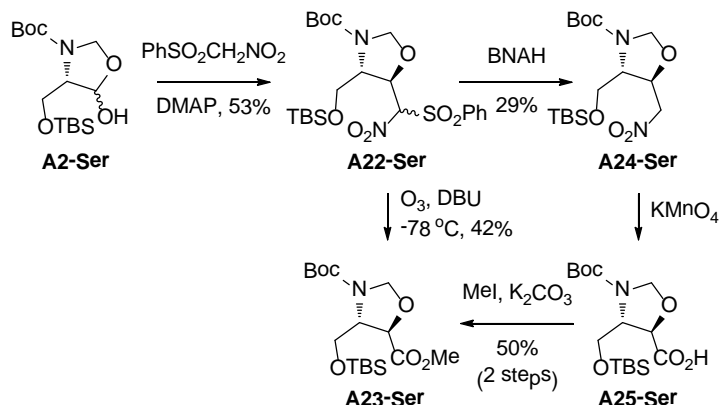


Scheme A12. The Henry reaction with the stable α -amino aldehyde.

To address the elimination issue of the *N*-hydroxymethyl group, probably caused by the basic conditions of the nitro-aldol reaction, we considered more reactive nitromethane derivative to produce the desired nitroolefin group in-situ via the facile dehydration of the initial nitro-aldol intermediate.

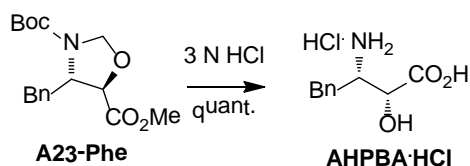
Among the reported activated nitromethanes,²⁵ phenylsulfonylnitromethane was well suited to our purpose. Initially, any of the desired nitro-aldol products were not produced with a dianion of phenylsulfonylnitromethane, prepared by treating phenylsulfonyl-nitromethane with LDA (3 equiv.) at low temperature, which was the reported reaction conditions by P. A. Wade *et al.*^{17b} Other bases such as BuLi, KHMDS, K₂CO₃, or Cs₂CO₃ did not help. Neither did use of a monoanion of phenylsulfonylnitromethane. Only starting materials were recovered intact after the reactions.

When the base was changed to TEA, however, some adduct with a phenylsulfonylnitromethane group was produced in low yield as a diastereomeric mixture. Among amine bases tested, such as TEA, DMAP, pyridine, *N*-methylmorpholine, and DBU, the highest yield (57%) of the diastereomeric adduct was obtained with DMAP. On the ¹H NMR spectrum of the adduct, no double bond seemed present. A downfield shift of the methine proton at around 5.5 ppm was typical for protons at the carbon of phenylsulfonylnitromethane, and its coupling pattern of doublet seemed to indicate the presence of the cyclized product **A22-Ser** (Scheme A13).



Scheme A13. β -Amino- α -hydroxy acids synthesis with $\text{PhSO}_2\text{CH}_2\text{NO}_2$.

Both structure and stereochemistry of the adduct **A22-Ser** were established after oxidation of the phenylsulfonylnitromethyl group of **A22-Ser** into the carboxylic ester group of **A23-Ser** at low temperature (-98 to -78°C),²⁶ where **A23-Ser** was obtained from **A22** in 85% yield (Scheme A13). No peaks from the minor isomer were seen on both GC and ^1H NMR charts of **A23-Ser**. Through the developed procedure, phenylalaninal derivative (**A23-Phe**) was also synthesized (Scheme A14). Finally, the stereochemistry of **A23-Phe** was confirmed after conversion into a known β -amino- α -hydroxy acid, (2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoic acid (AHPBA) as its HCl salt after global deprotection of the protecting groups of **A23-Phe** under acidic conditions (Scheme A14). Its spectral and physical data as well as the optical purity well matched those reported in the literature.²⁷



Scheme A14. The absolute stereochemistry of **A23-Phe**.

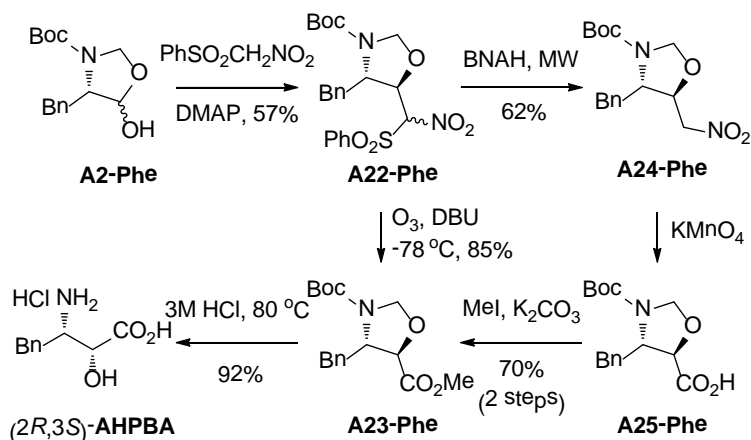
In summary, the application of *N*-hydroxymethyl group on a stable α -amino aldehyde had been expended to synthesize a series of β -amino- α -hydroxy acids. Although the high stereoselectivity had been observed via the intramolecular conjugate addition between the *N*-hydroxymethyl group and the introduced phenylsulfonylnitroolefin, which occurred with the addition of phenylsulfonyl-nitromethane to the stable aldehyde under basic reaction condition, the reaction conditions had to be optimized for better yield. If the reaction conditions were optimized and the reaction mechanism was able to be verified, the new application of *N*-hydroxymethyl group in the synthesis of β -amino- α -hydroxy acids could be utilized to synthesize biologically active compounds, such as bestatin and L-TBOA.

Results and Discussions

1. The stereoselective synthesis of *threo*- β -amino- α -hydroxy acids

The stereoselective synthesis of *threo*- β -amino- α -hydroxy acids had progressed via the intramolecular conjugate addition of the *N*-hydroxymethyl group on stable α -amino aldehydes. High stereoselectivity (>20:1) was obtained by the intramolecular conjugate addition of the *N*-hydroxymethyl group. In order to utilize the new stereoselective method to synthesize biologically active natural product, the reaction conditions had to be optimized.

Phenylalaninal **A2-Phe** was selected as a starting material (Scheme A15), contrasted to the initial reaction that was started with serinal derivative **A2-Ser**. The condensation reaction between phenylalaninal **A2-Phe** and the activated nitromethane, phenylsulfonylnitromethane ($\text{PhSO}_2\text{CH}_2\text{NO}_2$), was performed in the presence of DMAP with vigorous stirring. However, it was hard to purify the intramolecular conjugate adduct **A22-Phe** from the remained starting material due to their similar polarities. Moreover, the conjugate adduct showed the trailing on the silica gel chromatography. Therefore, the optimization on the condensation step was performed to obtain full conversion. The higher concentration of the reaction mixture, the more increased yield on the condensation step was observed, but the starting material had still remained. The addition of excess amount of phenylsulfonylnitromethane and base did not help to obtain full conversion.

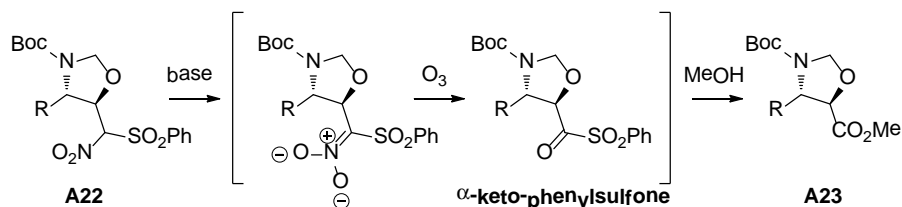


Scheme A15. Stereoselective synthesis of *threo*-AHPBA.

While the reaction conditions had been optimized, the structures of the synthesized compounds were reconfirmed by the functional group transformation and NMR studies. In the functional group transformation, the stereoselectivity of each compound was measured by ^1H NMR or gas chromatography.

The stereoselectivity of **A22-Phe** was measured right after the one-step conversion of phenylsulfonylnitromethyl group to methyl ester via ozonolysis at -78°C in methanolic solution. The *trans*-oxazolidine methyl ester **A23-Phe** was the sole product, which was confirmed by both ^1H NMR study and GC analysis. The proposed mechanism of the oxidative Nef reaction of phenylsulfonylnitromethane was depicted in Scheme A15. Under the basic reaction conditions, the conjugate adduct **A22** was converted to phenylsulfonylnitronate, and then the followed ozonolysis gave the corresponding α -keto-phenylsulfone. Finally, the α -keto phenylsulfon was

converted to the desired methyl ester **A23** via methanolysis.



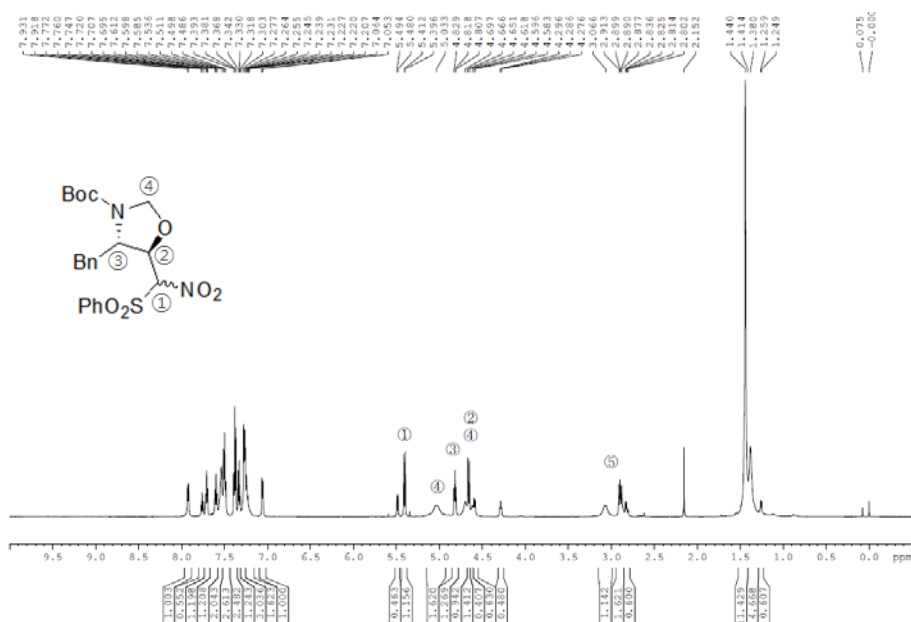
Scheme A16. Proposed mechanism of oxidative Nef reaction with **A22**.

The transformation of phenylsulfonylnitromethyl group on **A22-Phe** to nitromethane **A24-Phe** was another way to prove the stereoselectivity of **A22-Phe**, which was a diastereomeric mixture with approximately 2:1 ratio calculated based on ^1H NMR spectrum. Even under microwave assisted reaction conditions, the minor isomer was not detected both GC and ^1H NMR charts of **A24-Phe**. Moreover, much improved yield was obtained in the presence of AIBN under the microwave irradiation, instead of the exposure to UV lamp which was the original reaction conditions with **A22-Ser**. The following two-step transformation of **A24-Phe** into **A23-Phe** via **A25-Phe** also supported the stereoselective formation of **A22-Phe**.

The stereochemistry of **A23-Phe** was assumed as *trans*-oxazolidine based on the previous results on the conjugate addition of the *N*-hydroxymethyl group to the α,β -unsaturated ester group where the *trans*-oxazolidines were resulted from the favored *H*-eclipsed allylic conformation at the transition state.^{6-10, 28} The NMR coupling constants of the oxazolidine ring of **A23-Phe** was not useful to determine its relative stereochemistry because of the flexible conformation of **A23-Phe**. Finally, the stereochemistry of **A22-Phe** was

confirmed right after the acidic hydrolysis of **A23-Phe** by the comparing the spectral and physical data including the optical purity of the synthesized AHPBA with those reported in the literature.

Figure A4 showed ^1H NMR data of **A22-Phe** that was measured at 40 °C in order to make the peaks sharp. Not much of sharpening in the shape was observed even at 60 °C. As like the previously mentioned, α -proton of phenylsulfonylnitromethane was shown in 5.40~5.49 ppm. Because **A22-Phe** was the diastereomeric mixture, the determination of the peaks from major diastereomer was required to verify the structure. At the first step on the assignment of the peaks, decoupling studies had been tried to verify the peaks of the major diastereomer, but they were not clearly specified due to the similar chemical shifts between the two diastereomers.



Therefore, the heteronuclear single quantum coherence spectroscopy study of **A22-Phe** had been performed, and the observed spectra were shown in Figure A5 and A6. The verification of phenyl peaks from the major diastereomer was impossible. The α -protons of the major and minor diastereomers were clearly verified and the protons at ③ (shown in figure A5) were also verified although the chemical shift of them were quite different. However, other peaks, the protons at ② and ④ in figure A5, were not able to be determined due to the almost same chemical shifts and flexible conformation of the oxazolidine. From the results on HSQC studies and the previously mentioned decoupling studies (not shown) the assignment of **A22-Phe** was possible.

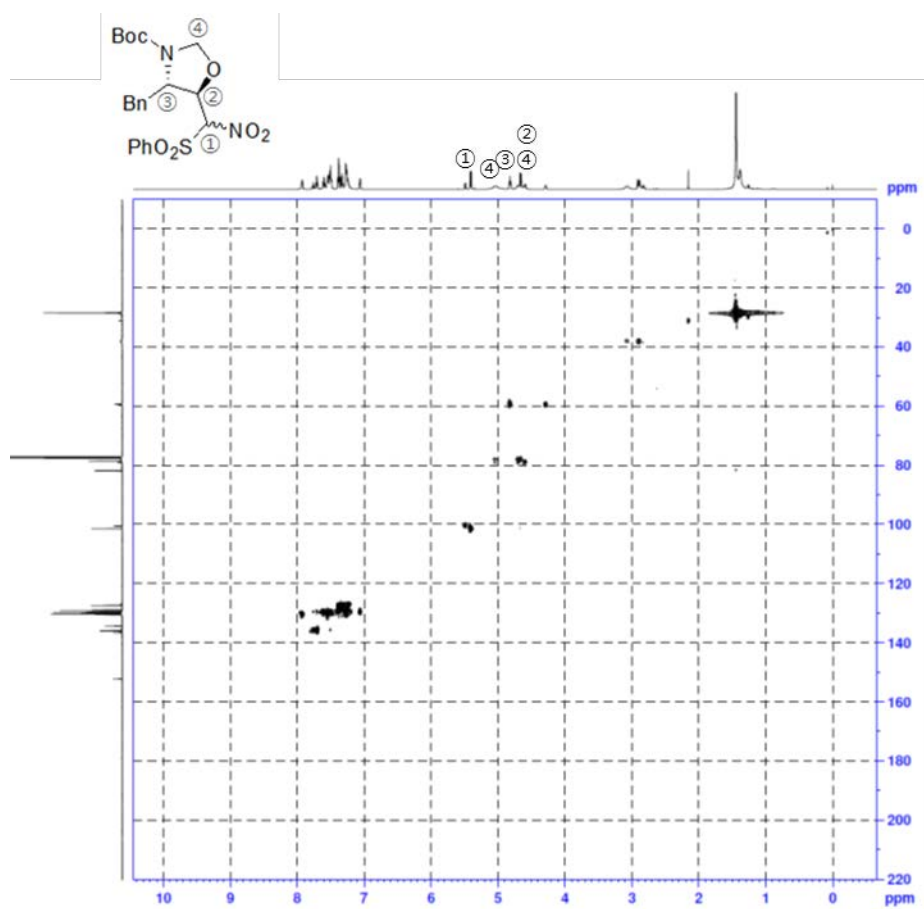


Figure A5. The spectrum of HSQC of **A22-Phe**.

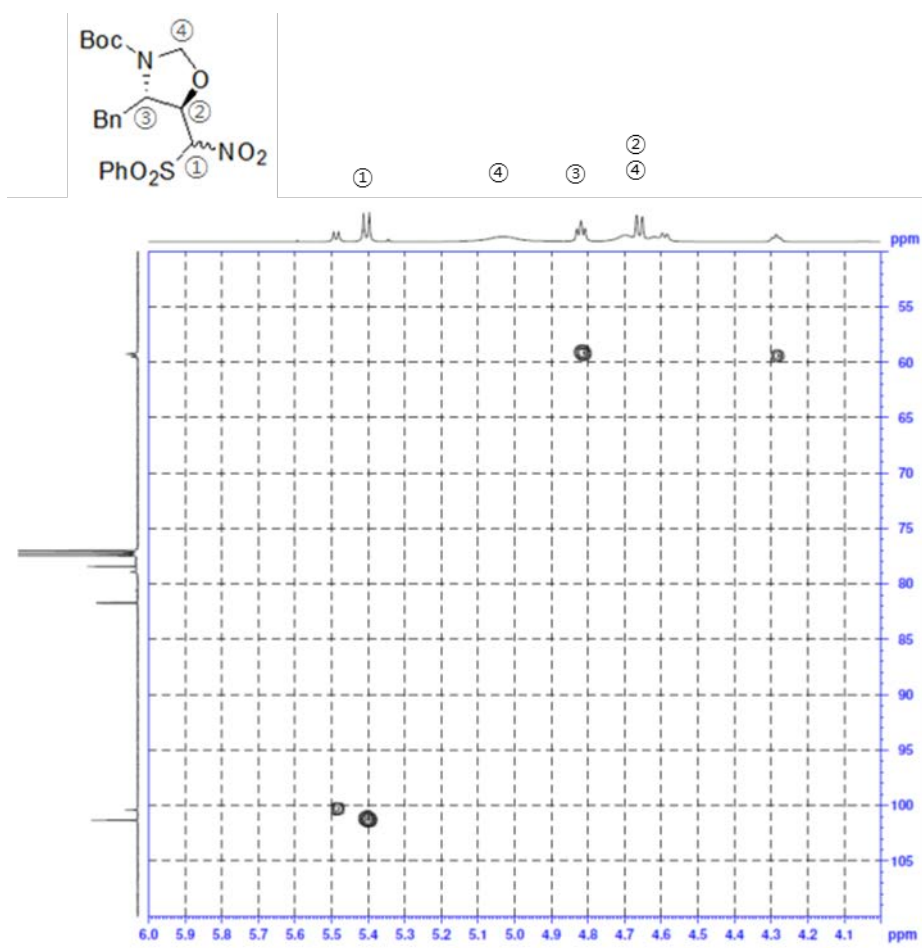
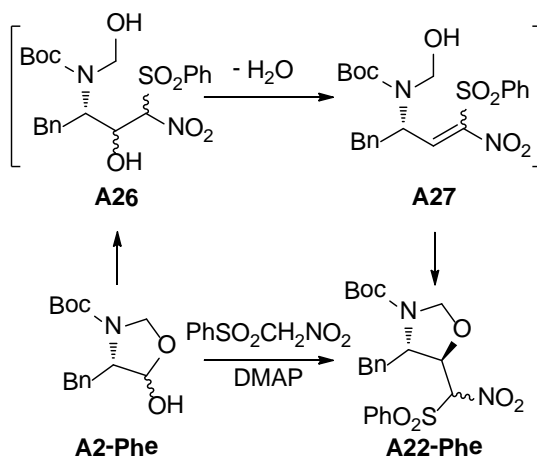


Figure A6. The expanded spectrum of HSQC of **A22-Phe**.

Based on the structural investigation of **A22-Phe**, it was proposed that the desired oxazolidine **A22-Phe** was produced probably from an intramolecular conjugate addition of the *N*-hydroxymethyl group to the activated nitroolefin of **A27**, which seemed to be generated in turn by facile dehydration of the resulting nitro-aldol intermediate **A26** (Scheme A17). The possibility of the direct cyclization of **A26** into **A22** was excluded based on the basic reaction conditions, under which an S_N2 reaction between the *N*-hydroxymethyl group and the β -hydroxy group of **6a** seemed difficult.

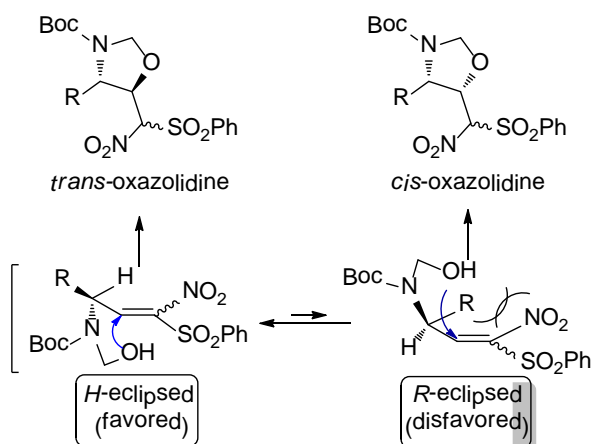


Scheme A17. Proposed mechanism of the intramolecular conjugate addition.

The three tandem reactions from **A2-Phe** to **A22-Phe**, i.e., the nitro-aldol, the dehydration, and the intramolecular conjugate addition reactions, occurred in one-pot (Scheme A17). Other points to note here were that the dehydration reaction from **A26** to **A27** did not require any additives, such as acids or leaving groups, and that the separate conjugate addition step from **A27** to **A22-Phe** was not required, which was necessary in the previous studies for the syntheses of γ -amino- β -hydroxy acids.⁶

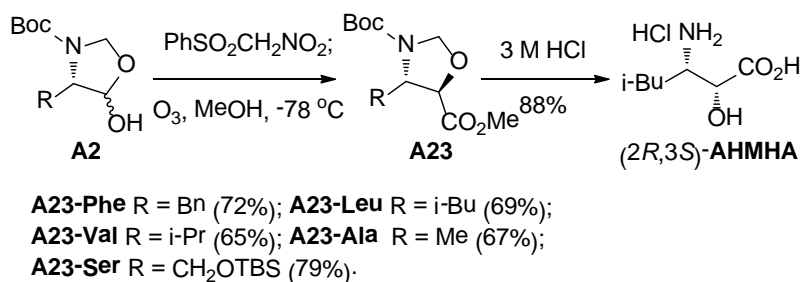
A high stereoselectivity seemed to be achieved in the intramolecular conjugate addition of **A27** into **A22-Phe** because only the two isomers out of the four possible isomers were notable in ^1H NMR spectrum of **A22-Phe** (Figure A4). Absence of the minor *cis* isomer on both GC and ^1H NMR charts of **A23-Phe** suggested that an excellent selectivity (more than 20:1) was attained in the intramolecular conjugate addition of the *N*-hydroxymethyl group of **A27** to give the *trans* isomer as nearly one isomer.

The selectivity for the *trans*-oxazolidine could be explained based on the previous reports about the intramolecular conjugate addition of the *N*-hydroxymethyl group to the α,β -unsaturated ester group.⁶ The much improved selectivity in this study may be attributed to the considerably favored *H*-eclipsed conformation at the transition state because of the increased allylic ($A^{1,3}$) strain from the bulkier *cis* substituent on phenylsulfonylnitroolefin (either SO_2Ph or NO_2) in the *R*-eclipsed conformation (Scheme A18).²⁸ The similar enhanced selectivity was previously observed in the stereoselective synthesis of *threo*- β -hydroxy-L-glutamic acid when the stereochemistry of the conjugated ester group was changed from the *E*- to *Z*-olefin.¹⁰



Scheme A18. Proposed transition state for high stereoselectivity.

Efficiency of the transformation process of **A2** into **A23** could be improved further through the in-situ ozonolysis of the diastereomeric adduct **A22** without its isolation. Thus, the *trans*-oxazolidine methyl ester **A23** was produced in 72% yield from α -amino aldehyde **A2-Phe** from the one-pot process (Scheme A19), which gave a much higher yield than that of the stepwise process in Scheme A15 (overall 48% yield).



Scheme A19. β -Amino- α -hydroxy acids from the one-pot process.

The one-pot process had been also successfully applied to other α -amino aldehydes, which were derived from the commercially available α -amino acids (Scheme A19). The *trans*-oxazolidine methyl esters of β -amino- α -hydroxy acids **A23** were obtained in 65% to 79% yields with more than 20:1 stereoselectivity by following the one-pot procedure. Both structure and stereochemistry of **A23** were assigned as an analogy to that of **A23-Phe**.

Most of the reaction conditions for other α -amino aldehydes were the same as those for the phenylalaninal **A2-Phe** as described above except for the valinal derivative **A23-Val**, which required higher reaction temperature of 50 °C instead of room temperature on the condensation with phenylsulfonylnitromethane. Interestingly, the valinal **A2-Val** also showed the high stereoselectivity of the *trans*-oxazolidine, which was a different result from the previous studies with the valinal derivative. In the previous study on the intramolecular epoxidation with the *N*-hydroperoxymethyl group, the opposite stereoselectivity, *syn*-epoxide, was observed with valinal derivatives due to the A^{1,3}-strain whereas serinal derivative converted to the corresponding *anti*-epoxide via the favorable N-eclipsed allylic conformation from the dipole-dipole repulsion between the peroxy group and the double bond.⁸

With leucinal **A2-Leu**, another well-known *threo*- β -amino- α -hydroxy acid, (2*R*,3*S*)-3-amino-2-hydroxy-5-methylhexanoic acid, (2*R*,3*S*)-AHMHA, was prepared in 88% yield as a HCl salt by acidic hydrolysis of **A23-Leu**, whose spectral and optical data were nearly identical with its enantiomer in the literature (Scheme A19).²⁹

In summary, a novel and efficient synthetic method for β -amino- α -hydroxy

acids and their derivatives had been developed with the *N*-hydroxymethyl protected α -amino aldehydes. Desired β -amino- α -hydroxy acid derivatives **A23** were produced with high stereoselectivity in a one-pot process from the reactions of the α -amino aldehydes and phenylsulfonylnitromethane in the presence of an amine base followed by in-situ oxidation. Thus, protected β -amino- α -hydroxy esters **A23** were prepared in 65% to 79% yields with more than 20:1 stereoselectivity in one reaction flask. Both absolute and relative configuration of **A23-Phe** and **A23-Leu** were established beyond doubt after one-step acidic hydrolysis into the known β -amino- α -hydroxy acids, (2*R*,3*S*)-AHPBA and (2*R*,3*S*)-AHMHA as their HCl salts, respectively. A probable reaction mechanism for the three tandem reactions, i.e., the nitro-aldol, the dehydration, and the intramolecular conjugate addition reactions, was proposed to rationalize the reaction results.

Based on the well-developed stereoselective synthetic method for β -amino- α -hydroxy acid, some biologically active natural products had been synthesized. The detail results would be presented in the following chapters.

2. Application of the stereoselective synthetic method

for β -amino- α -hydroxy acids : bestatin and its derivatives

Bestatin, also known as ubenimex, had been explored as a potent peptidase inhibitor, especially as an aminopeptidase N (APN) inhibitor. Generally, APN releases N-terminus neutral amino acids from oligopeptide or protein via the activated hydrolysis of the peptide bond by the coordination with Zn^{2+} on the active site of aminopeptidase N.¹¹ The effects of APN on the protein activation, degradation, and regulation were related with the inflammatory diseases and tumors. For this reason, APN inhibitor had been studied for therapeutic means. Among the known APN inhibitors, bestatin showed multiple effects on the immune system with low toxicity.³⁰ Its structure was known as a dipeptide of the unnatural vicinal amino hydroxy acid at the N-terminus and the natural leucine amino acid at the C-terminus (Figure A7).

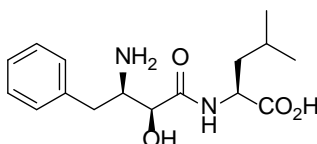


Figure A7. The structure of bestatin.

The interaction between bestatin and the Zn^{2+} embedded at the active site of aminopeptidase was completed by the participation of its free amino, hydroxy and carbonyl group of peptide bond (Figure A8).³¹

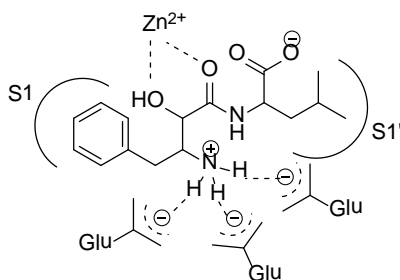


Figure A8. The interaction between bestatin and the active site of APN.

Based on the diverse effects of bestatin on immune system, various synthetic methods had been reported for the stereoselective synthesis of bestatin,³² and diverse analogs from epibestatin to leucine residue replacements had been synthesized and studied to identify their bioactivities. However, not many of studies had been done on the N-terminus residue modification.

According to the recent report on the human aminopeptidase N (hAPN), the carboxylic acid at C-terminus of bestatin interacted with Zn^{2+} at the active site of hAPN, and other functional groups, amino, hydroxy and the carbonyl group at the peptide bond, made hydrogen bonds with the glutamine at the active site (Fig. A9). Dissimilar to the interaction of bestatin, β -amino- α -hydroxy acid unit on amastatin interacted with Zn^{2+} on hAPN (Fig. A10). The different interactions on hAPN may be caused by the side chains on the aminopeptidase inhibitors. In other words, the benzyl group on bestatin formed a π - π interaction with the phenylalanine at hAPN, which made a different complex pattern with amastatin.

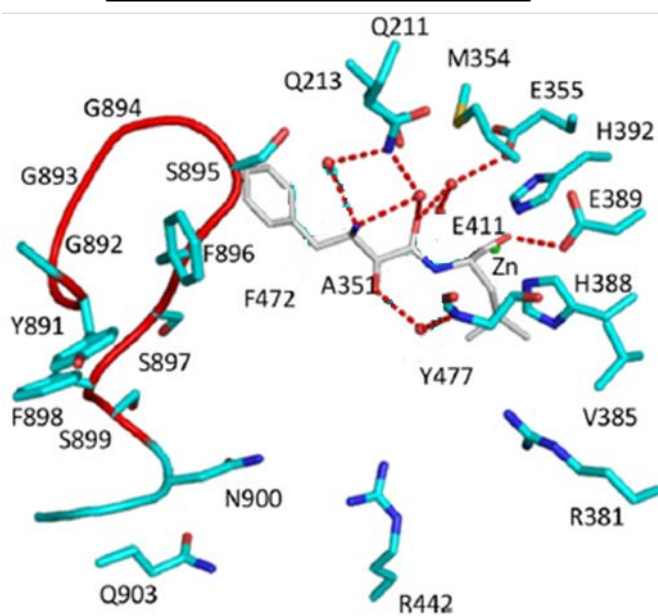
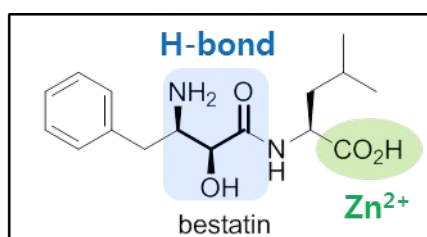


Figure A9. The interaction of bestatin with hAPN.

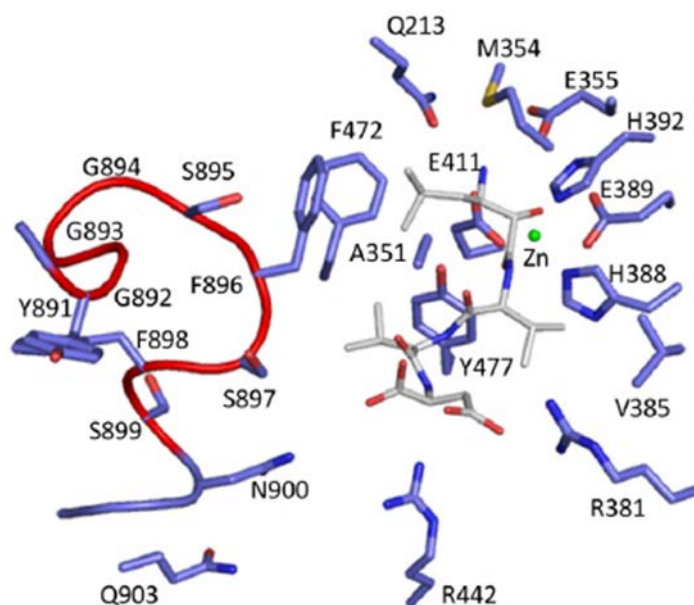
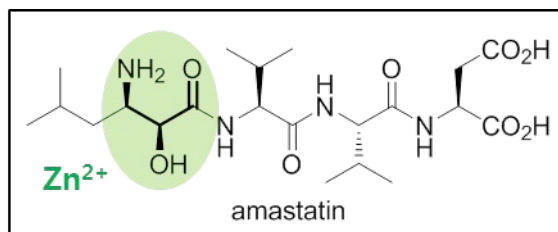
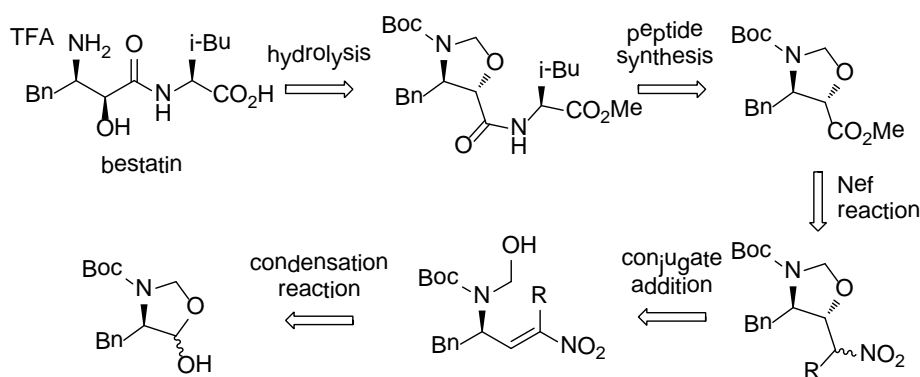


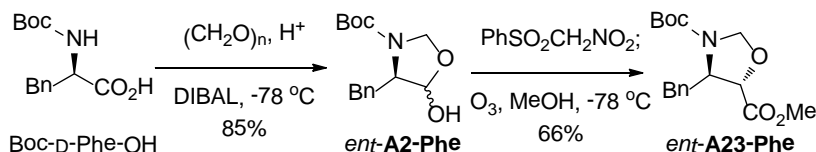
Figure A10. The interaction of amastatin with hAPN.

As the first application of the developed stereoselective synthetic method for *threo*- β -amino- α -hydroxy acids, the synthesis of bestatin had been planned (Scheme 19). Because the intramolecular conjugate addition of the *N*-hydroxymethyl group could be applied to the various amino acids, new bestatin analogs containing alkyl groups instead of benzyl group at the N-terminus was also planned to be synthesized with the stereoselective intermediate *trans*-oxazolidine methyl esters.



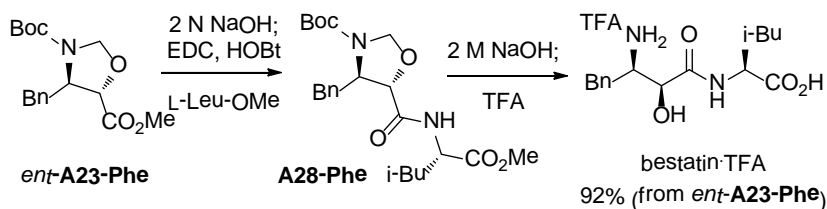
Scheme 20. Retrosynthesis of bestatin.

The synthesis of bestatin was started with the preparation of the suitable starting material, enantiomer of **A2-Phe**. From commercially available *N*-Boc-D-phenylalanine, the stable α -amino aldehyde had been synthesized via the same procedure of the previously reported paper (Scheme 21),⁸ and the stable α -amino aldehyde had been converted to the corresponding stereoselective methyl ester *ent*-**A23-Phe** according to the one-pot protocol as shown in Scheme 19.



Scheme A21. Preparation of the starting material for bestatin synthesis.

A simple peptide coupling of free carboxylic acid (not shown), which produced from the basic hydrolysis of *ent*-**A23-Phe**, with L-leucine methyl ester (L-Leu-OMe) afforded peptide **A28-Phe**, and then the followed basic and acidic hydrolysis gave bestatin as its TFA salt (Scheme 22). Finally, a known dipeptide bestatin was obtained in high yield (92%) without the separate protection and/or deprotection steps for the vicinal amino hydroxy acids moiety. The spectral and physical data of the synthesized bestatin were identical with those in the literature.

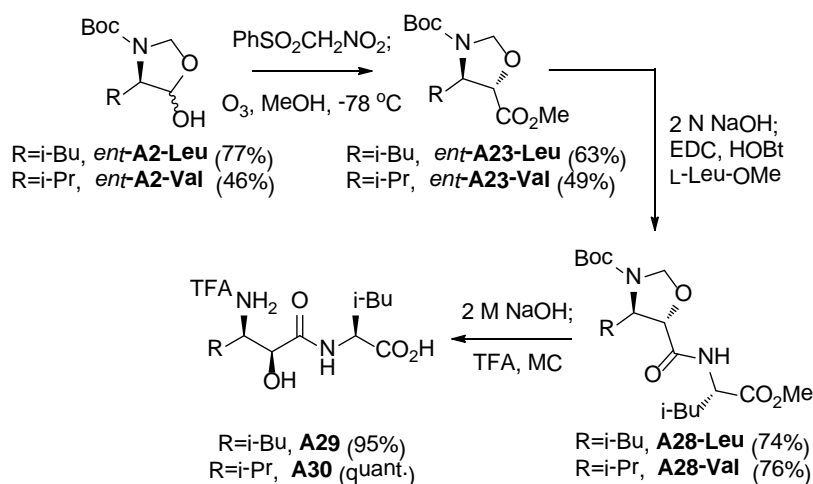


Scheme 22. Synthesis of bestatin.

One of the bestatin analogs,² AHPBA-Val, was also synthesized by following the same synthetic process (Scheme A22), which was reported to be a more potent aminopeptidase inhibitor than bestatin.³³

Based on the successful synthesis of bestatin and AHPBA-Val, bestatin analogs that possess alkyl groups at the N-terminus had been synthesized via

the same procedure. One of the synthesized bestatin analogs was started with *N*-Boc protected D-leucine, while the other analog was synthesized with *N*-Boc protected D-valine. Each of the α -amino aldehyde, lactol, was converted to the stereoselective methyl ester *ent*-**A23-Leu** or *ent*-**A23-Val** in 63% or 49% yield, respectively. Unfortunately, the yield of *ent*-**A23-Val** had not increased even in extended reaction time at 40 or 50 °C. In the peptide bond synthesis, isobutyl chloroformate was applied at first, the desired peptide was synthesized in less than 50% yield. In order to optimize the reaction conditions, the coupling reagent was changed to *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbonate (EDC), and the desired peptides had been synthesized in better yield (74%) in the presence of HOBt (Scheme A23).



Scheme A23. Synthesis of betatin analogs, **A29** and **A30**.

During the previous synthesis of bestatin and AHPBA-Val, the final compounds sometimes were obtained with undefined impurities. Therefore, the

reaction conditions on the acidic hydrolysis for the deprotection of *N*-Boc group and *N,O* acetal cleavage had been changed. The desired bestatin analogs had been produced without impurities via the simple extraction with water and ethyl acetate (EtOAc) after the acidic hydrolysis in methylene chloride solution.

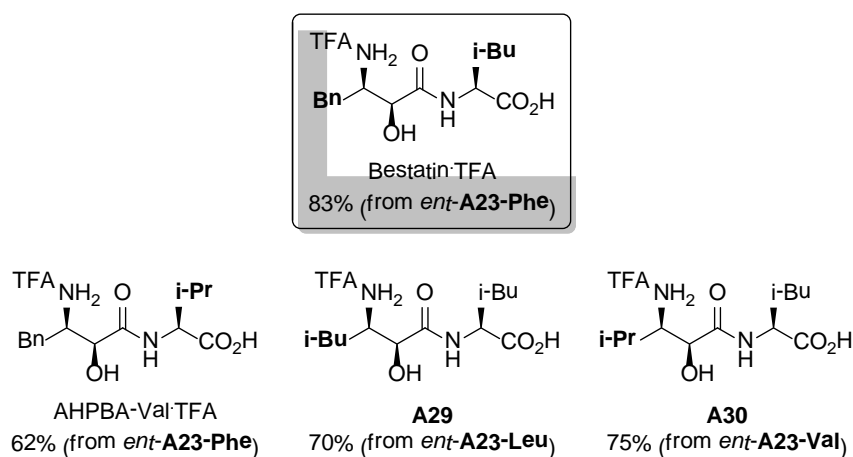


Figure A11. The stereoselectively synthesized dipeptides.

In summary, the well-known aminopeptidase inhibitor, bestatin, which was also known as Ubenimex, had been synthesized from the *trans*-oxazolidine methyl ester (*ent*-**A23-Phe**). Because the applied oxazolidine was a protected form of the vicinal amino alcohol, the protection (or deprotection) of the functional groups was not required. Moreover, its analogs were stereoselectively synthesized from *N*-Boc-D-amino acids effectively via the well-developed procedures. Among the synthesized analogs, **A29** and **A30** were synthesized for the first time.

3. Application of the stereoselective synthetic method

for β -amino- α -hydroxy acids : *threo*- β -benzyloxyaspartate

L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system, and its action is thought to be linked to higher brain functions, such as memory and learning. However, the extracellular concentration of it is strictly controlled at $\sim 1 \mu\text{M}$ because glutamate is also known as a potent excitotoxin that causes neuronal degeneration.² The control of the concentration is performed by glutamate transporters. Although their important roles have been elucidated by genetic approaches, genetic manipulations cannot provide any information on the dynamics of glutamate homeostasis in response to the acute disruption of uptake systems. Therefore, pharmacological intervention with selective blockers is an essential approach to investigate the physiological roles of the transporters.

Many glutamate analogs had been screened as uptake inhibitors, and they had been important tools to address the function of glutamate transporters.³⁴ In addition, conformationally constrained analogs had provided useful pharmacophore models, but most of these inhibitors acted as competitive substrates, which were transported. Although such competitive substrates reduced glutamate uptake, they induced ion flux or heteroexchange, which was the exchange of external substrate with internal substrate. Therefore, non-transportable blockers that did not affect glutamate receptor were indispensable for elucidating the function of the glutamate transporters.

Sometimes a substrate for the glutamate transporters was converted to a blocker. For example, L-aspartate (L-Asp) was a high-affinity substrate for the

transporters,³⁵ but it was converted as a blocker by introducing substituents at the β -position. The introduced β -hydroxy group may decrease both transportability through transporters and affinity toward glutamate receptors (Figure A10).¹²

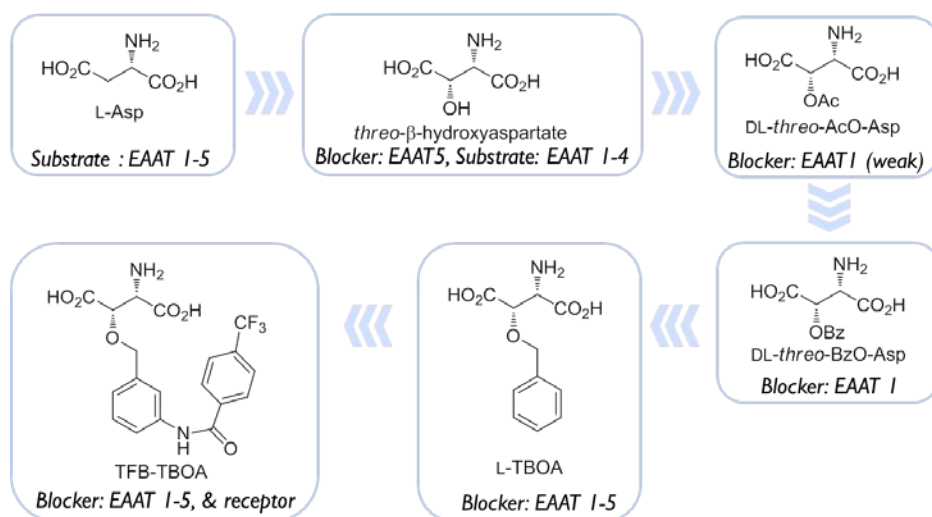
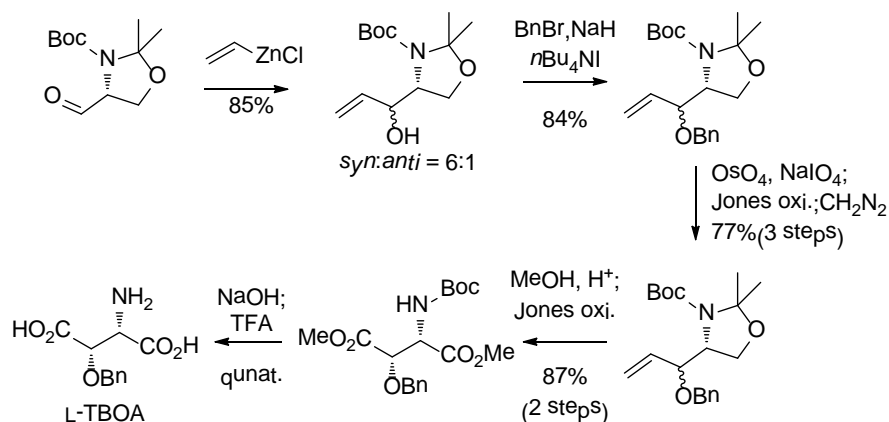
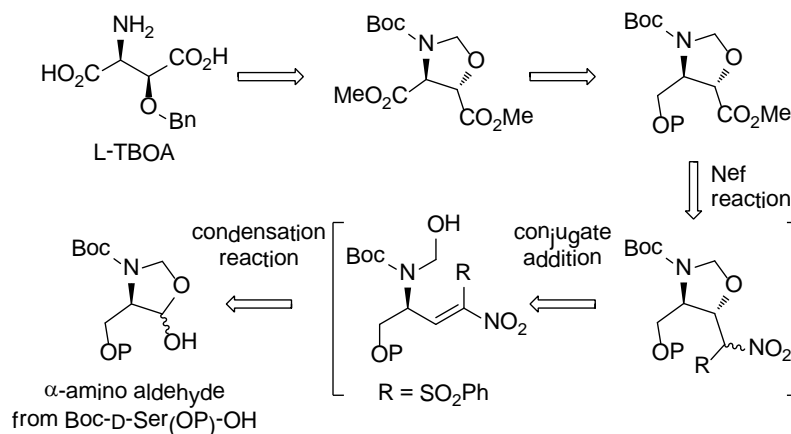


Figure A12. The conversion of L-Asp as the glutamate blocker.

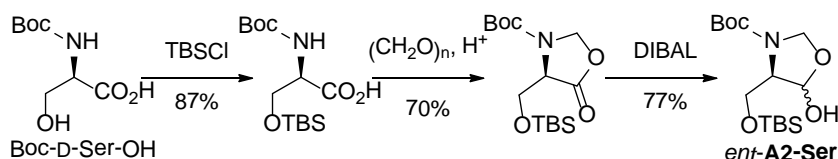
While glutamate uptake was inhibited by *O*-acetylated THA,³⁵ *O*-benzoyl analog did not only inhibit the uptake but also decreased the glutamate induced current. Due to the unstability of *O*-acyl analogs, however, ether-type analogs were synthesized. Among the ether-type analogs, *L*-threo- β -benzyloxyaspartate (L-TBOA), a specific extracellular amino acid transporter (EAAT) antagonist,³⁸ showed the inhibitory activity against glutamate transporters.³⁹ Therefore, the syntheses of *L*-threo- β -benzyloxy aspartate and its derivatives had been reported by Shimamoto group.⁴⁰



In the previous synthesis of L-TBOA, the corresponding vicinal amino alcohol had been synthesized in moderate stereoselectivity (*threo:erythro* = 6:1) via the addition of vinyl zinc chloride to (*R*)-Garner aldehyde (Scheme A23). The moderate stereoselectivity and efficiency on the synthesis of L-TBOA was attractive because those could be improved via the intramolecular conjugate addition of the *N*-hydroxymethyl group. The scheme A25 depicts the brief plan for the stereoselective synthesis of L-TBOA.



At the beginning on the synthesis of L-TBOA, the suitable amino acid was selected as D-Ser-OH, which possesses hydroxymethyl side chain. The side chain could be readily converted to methyl ester moiety by the reported procedures including deprotection, the oxidation and the following methylation.¹⁰ Therefore, the stable α -amino aldehyde (*ent*-**A2-Ser**) with *N*-hydroxymethyl group was prepared by following the previous procedures.¹⁰

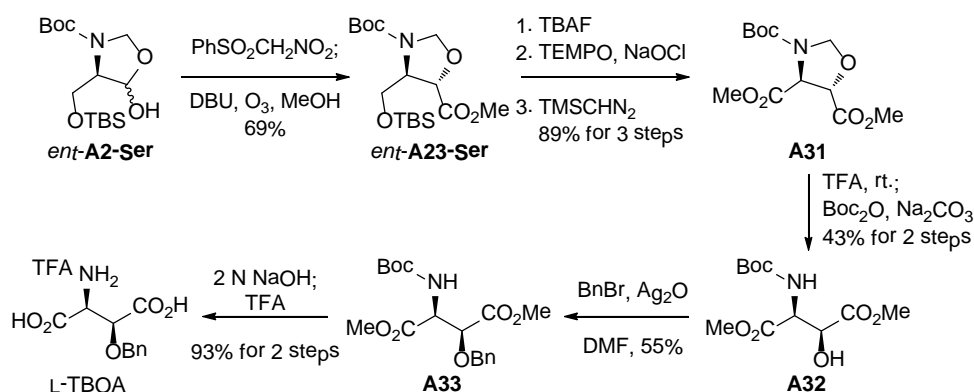


Scheme A26. The preparation of the stable serinal derivative.

The same one-pot procedure (Scheme A19) was applied for the highly stereoselective synthesis of *ent*-**A23-Ser** (Scheme A26). The desired *trans*-oxazolidine methyl ester *ent*-**A23-Ser** was produced in 69% yield with more than 20:1 stereoselectivity in one reaction vessel, where the tandem three reactions occurred between the serinal derivative *ent*-**A2-Ser** and the activated nitromethane $\text{PhSO}_2\text{CH}_2\text{NO}_2$. The high stereoselectivity was presented by the intramolecular conjugate addition between the *N*-hydroxymethyl group and the temporarily generated phenylsulfonyl nitroolefin.

With the *trans*-oxazolidine methyl ester *ent*-**A23-Ser**, the transformation of hydroxymethyl group was performed to synthesize *trans*-oxazolidine dimethyl ester **A31**. First, the silyl protecting group was removed with TBAF, and the primary alcohol was oxidized to the corresponding carboxylic acid.⁴¹

After the reaction with TMS diazomethane, acidic hydrolysis with excess amount of TFA in CH_2Cl_2 solution accompanied (2*S*, 3*S*)-dimethyl-2-amino-3-hydroxysuccinate (not shown). The impurities from the acidic hydrolysis were removed by the simple extraction with water and ethylene acetate. In order to obtain the *N*-Boc-protected form of dimethyl-2-amino-3-hydroxysuccinate, several reaction conditions with some reagents including oxalic acid were applied to cleavage of the *N*, *O* acetal, but the desired product was not obtained. Unfortunately, the previously reported oxidation of the *N*, *O* acetal with RuCl_3 ^{7,10} did not present the corresponding product (not shown). Therefore, the acidic hydrolysis with TFA and *N*-Boc protection were schemed although the efficiency of the synthesis was impaired by the additional protection step.



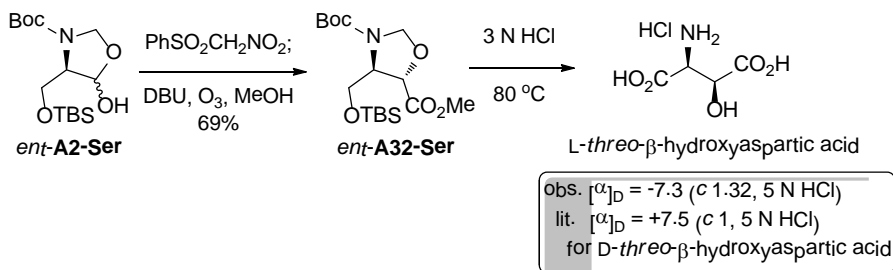
Scheme A27. The stereoselective synthesis of L-TBOA.

Before the introduction of benzyl group to β -hydroxy group on **A32**, the free amino group was protected with Boc. In the protection, hydrolyzed carboxylic acid was obtained with NaHCO_3 and Boc_2O under the ambient atmosphere. When the reaction was operated under the anhydrous conditions with Na_2CO_3

at room temperature, Boc protection was arisen at both amino and hydroxyl groups. Due to the formation of the di-Boc protected compound (not shown), the yield on the Boc protection was obtained in below 50%.

With the desired *N*-Boc protected dimethyl succinate **A32**, the protection of the hydroxyl group with benzyl bromide was performed under the basic reaction conditions. After the following sequential basic and acidic hydrolysis, the desired *threo*- β -benzyloxy aspartic acid was synthesized with high stereoselectivity.

The stereochemistry was verified via the formation of *L-threo*- β -hydroxy aspartic acid by the acidic hydrolysis of *ent*-**A32-Ser** (Scheme A28). The measured optical rotation on the prepared *L-threo*- β -hydroxy aspartic acid was shown in scheme A28 with the previously reported value, which was measured with the enantiomer.



Scheme A28. The stereochemistry of *ent*-**A32-Ser**.

Finally, the stereoselective synthesis of *L-threo*- β -benzyoxyaspartic acid, another application of the stereoselective synthetic method for β -amino- α -hydroxy acids with the *N*-hydroxymethyl group, was completed via the intramolecular conjugate addition of the *N*-hydroxymethyl to the temporary

generated phenylsulfonylnitromethane. The successful development of the stereoselective route for L-TBOA would be contributed to the elucidation on the function of glutamate transporters through the facile derivatization of L-TBOA.

4. Application of the stereoselective synthetic method

for β -amino- α -hydroxy acids : lapstatin and its derivatives

Streptomyces species have been used as a source of low-molecular-weight enzyme inhibitors, and well-known aminopeptidase inhibitor bestatin, amastatin and AHPBA-Val are come from these species. Besides these three aminopeptidases, phebestin and actinonin are also originated from *Streptomyces* MG848-hF6 and *Streptomyces* sp. MJ716-m3 respectively.

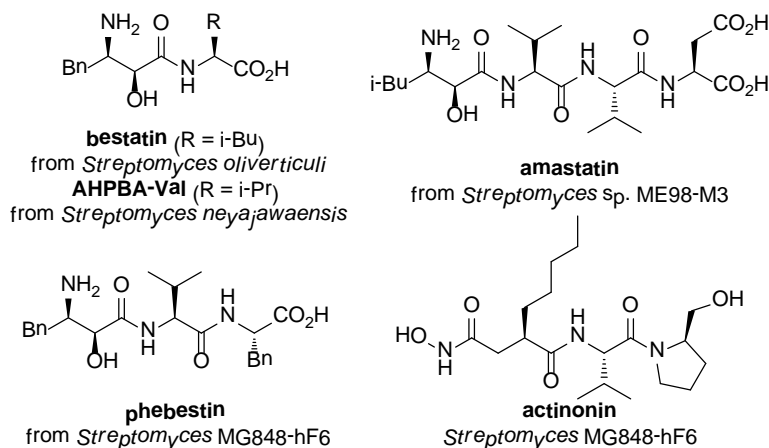


Figure A13. Aminopeptidase inhibitors from *Streptomyces*.

Since a leucine aminopeptidase was isolated from the waste broth of oxytetracycline production by *Streptomyces rimosus*, it was found that the leucine aminopeptidase was inhibited by bestatin and amastatin. Based on the previous studies on *Streptomyces rimosus*, it was curious that the culture medium of *S. rimosus* contained a low-molecular-weight inhibitor. Through the isolation and characterization, a new specific inhibitor of bacterial leucine

aminopeptidase had been reported. The new aminopeptidase had been named as lapstatin. Although the structure of it had been reported by Lampret *et al* in 1999,⁴² the stereochemistry had not been verified yet (Figure A14). According to the report, lapstatin contained natural amino acid valine and unnatural amino acid, 3-amino-2-hydroxy-4-methylpentanoic acid (AHMPA). The unnatural amino acid, AHMPA, was a β -amino- α -hydroxy acid that contained isopropyl group.

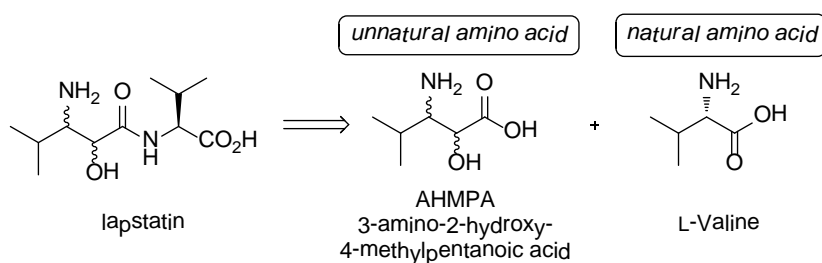
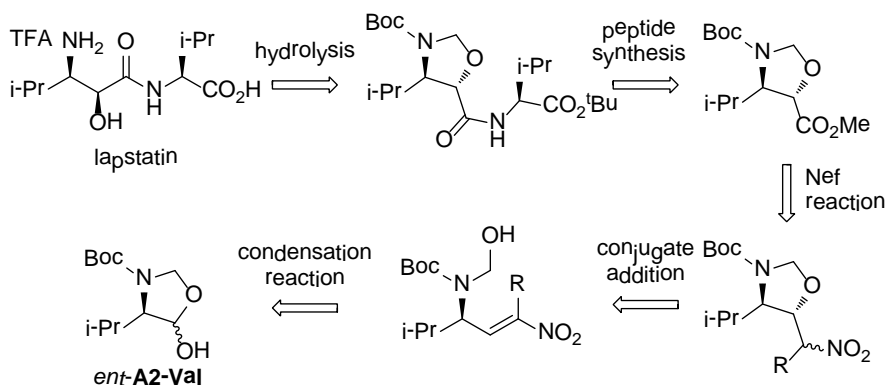


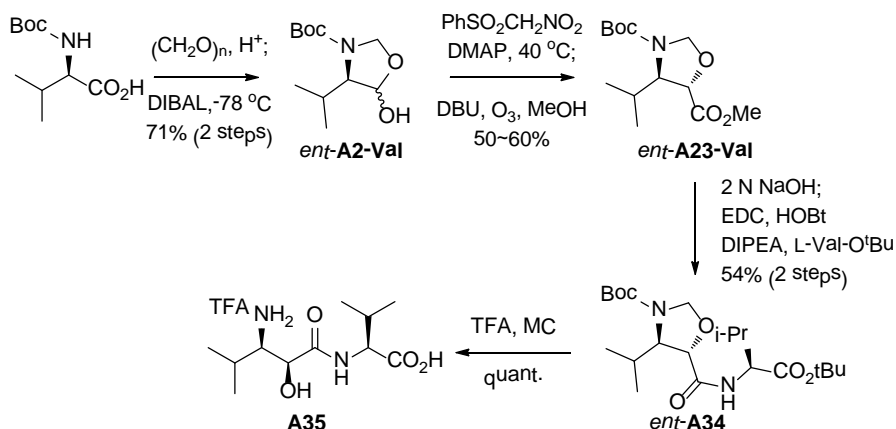
Figure A14. Structure of lapstatin.⁴¹

As like previously mentioned, the stereoselective intramolecular conjugate addition of the *N*-hydroxymethyl group could be started with various amino acids including phenylalanine, leucine and serine. The unnatural amino acid, AHMPA, could be synthesized from L-valine or D-valine. Therefore, the synthesis of lapstatin was planned to verify the stereochemistry of 3-amino-2-hydroxy-4-methylpentanoic acid (AHMPA) via the stereoselective intramolecular conjugate addition (Scheme A29).



Scheme A29. Retrosynthesis of lapstatin from D-Valine.

The synthetic plan for lapstatin is similar to the synthesis of bestatin derivatives (Scheme A22). At first, two stable α -amino aldehydes **A2-Val** were prepared from Boc-D-Val-OH, and Boc-L-Val-OH, and then the reaction with phenylsulfonylnitromethane was performed with each of the valinal derivatives (**A2-Val** or *ent*-**A2-Val**). Unfortunately, the full conversion of the valinal derivatives had not observed during the optimization of the reaction conditions, such as reaction temperature, solvent, or the concentration of the reaction mixture, and the disappointing results might be caused by the higher stability of the valinal derivatives than the other derivatives. Although the reaction condition had not been optimized, the rest of the synthesis was progressed in order to synthesize the dipeptides containing *threo*-AHMPA.



Scheme A30. Synthesis of lapstatin from Boc-D-Val-OH.

The in-situ oxidative Nef reaction via ozonolysis with the conjugate adducts **A22-Val** afforded the stereoselective intermediate *trans*-oxazolidine methyl esters **A23-Val**. The following basic hydrolysis and peptide coupling with L-Val-O^tBu produced two diastereomers. The desired dipeptides were finally obtained by the acidic hydrolysis with TFA. Figure A15 showed the synthesized dipeptides which containing *threo*-AHMPA at N-terminus and L-Valine at C-terminus.

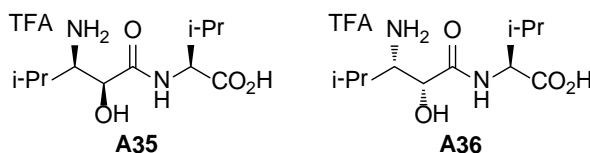
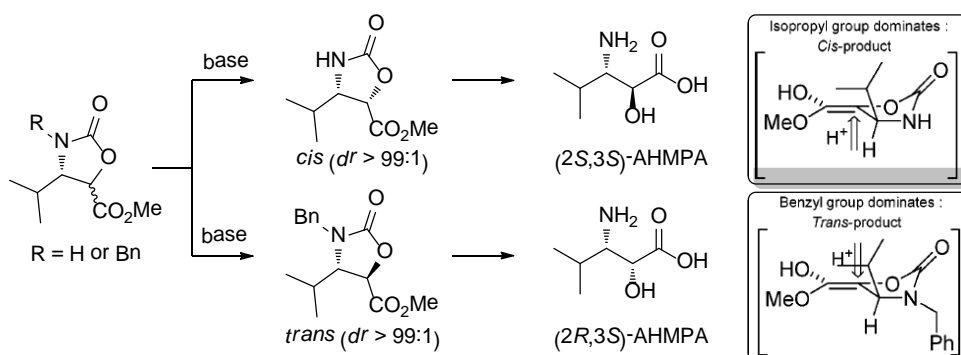


Figure A15. The prepared dipeptides **A35** and **A36**.

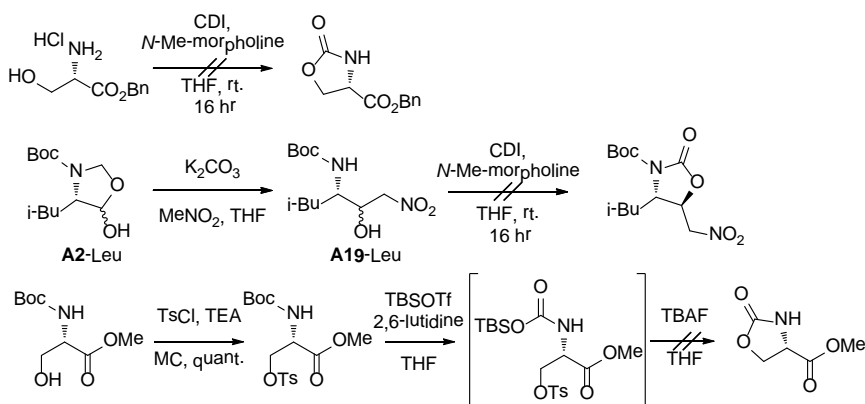
¹H NMR spectra of **A35** was almost identical with the reported data.⁴² However, it was not enough to conclude that the lapstatin contained (2*R*,3*S*)-

AHMPA because of the lack of other analytical informations, such as optical rotation, from the previous report. Therefore, the synthesis of *erythro*-AHMPA had been tried. There was a report on the stereodivergent synthesis of β -amino- α -hydroxy isopentanoic acid via diastereoselective epimerization (Scheme A31).⁴³



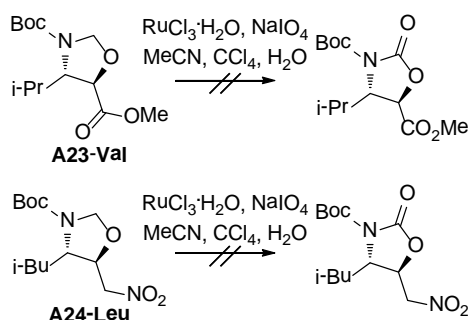
Scheme A31. Stereodivergent synthesis of β -amino- α -hydroxy acids.

In order to take the reported diastereoselective epimerization, oxazolidinone synthesis had been tried with protected serine or leucinal nitro aldol product **A19-Leu**, but the desired product had not been obtained (Scheme A32).



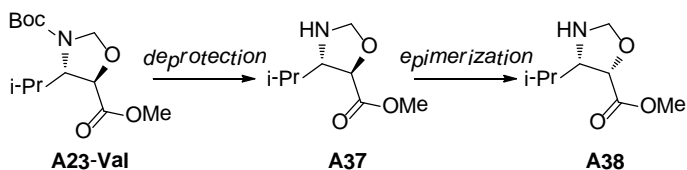
Scheme A32. Model study on carbocyclization.

Another attempt to prepare the oxazolidinone was the oxidation of the oxazoline with ruthenium catalyst. In the previous study on the divergent synthesis of γ -amino- β -hydroxy acids, the mixture of oxazolidone and *O*-formylated compound was obtained under the oxidation reaction conditions with RuCl_3 and *t*BuOOH. Unfortunately, the desired product was not obtained with **A23-Val**. It was assumed that high stability of the valine derivative may cause the disappointed result. However, the desired oxazolidinone had not been obtained even with **A24-Leu**.



Scheme A33. Ruthenium catalyzed oxidation with **A23-Val** and **A24-Leu**.

Based on the disappointed results on the preparation of oxazolidinone, the epimerization of the oxazolidine **A23-Val** had been tried, and the possibility on the epimerization had been observed (Scheme A34).



Scheme A34. Epimerization of **A23-Val** for *erythro*-AHMPA synthesis.

The verification and optimization of the products from the epimerization with **A23-Val** might allow the synthesis of *erythro*-AHMPA. The studies on the synthesis of *erythro*-AHMPA had been progressed on our lab. The following two dipeptides containing *erythro*-AHMPA will be significant evidences to support the determined stereochemistry of lapstatin by comparing the reported NMR spectrum with that of the synthesized dipeptides **A35** and **A36**.

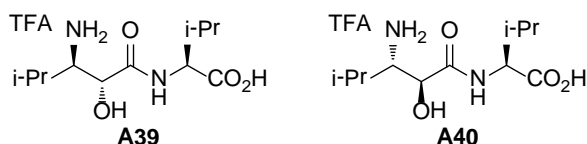


Figure A16. The prepared dipeptides with *erythro*-AHMPA.

As like mentioned at the beginning of this section, this was the first attempt of the verification of stereochemistry on lapstatin, which was known as low-molecular-weight enzyme inhibitors. The verification of the stereochemistry and the development of divergent synthesis of β -amino- α -hydroxy acid would be used for the preparation of diverse aminopeptidase inhibitors, which had been used for the biochemical characterization of aminopeptidases and for studies of their active-site structures and catalytic mechanisms.

Conclusion

It had been developed that a novel and efficient synthetic method for β -amino- α -hydroxy acids and their derivatives from the *N*-hydroxymethyl protected α -amino aldehydes. Desired β -amino- α -hydroxy acid derivatives **A23** were produced with high stereoselectivity in a one-pot process from the reactions of α -amino aldehydes with the *N*-hydroxymethyl group and phenylsulfonylnitromethane in the presence of an amine base followed by in-situ oxidation. From the developed stereoselective intramolecular conjugate addition, several bioactive compounds including bestatin and L-TBOA had been synthesized. Moreover, the verification of stereochemistry of lapstatin had been progressed for the first time.

Experimental Details

General Procedure. Materials were obtained from commercial suppliers and were used without further purification. Methylene chloride was distilled from calcium hydride immediately prior to use. Air or moisture sensitive reactions were conducted under nitrogen atmosphere using oven-dried glassware and standard syringe/septa techniques. The reactions were monitored with a SiO₂ TLC plate under UV light (254 nm) and by visualization with a ninhydrin staining solution. A CEM Discover SP microwave unit was used for microwave reactions. In the microwave reactions, reaction temperature and pressure were monitored by the provided monitoring software. Column chromatography was performed on silica gel 60 (70-230 mesh). Optical rotations were determined at ambient temperature with a digital polarimeter and are the average of ten measurements. ¹H and ¹³C NMR spectra were measured at 400 MHz and 100 MHz, respectively in CDCl₃ unless stated otherwise. The ¹H NMR spectral data were reported as follows in ppm (δ) from the internal standard (TMS, 0.0 ppm): chemical shift (multiplicity, integration, coupling constant in Hz). The ¹³C NMR spectra were referenced with the 77.16 resonance of CDCl₃, 39.51 resonance of DMSO-*d*₆, or 49.00 resonance of MeOH-*d*₄. Gas chromatographic analyses were done with a capillary column (30 m × 0.25 mm). Infrared spectra were obtained as a film on a SiO₂ wafer. Low and high resolution mass spectra were measured by the CI ionization method and analyzed by magnetic sector mass analyzer.

General procedure for an adduct A22 from A2

To α -amino aldehyde **A2-Phe** (R = Bn, 230 mg, 0.82 mmol) in THF (1 mL) was added phenylsulfonylnitromethane (199 mg, 0.90 mmol) and DMAP (151 mg, 1.24 mmol). The mixture was stirred at room temperature for 2 days until the starting material **A2-Phe** disappeared. The reaction mixture was diluted with EtOAc (5 mL), to which was added an aqueous saturated solution of NH₄Cl (20 mL). The resulting mixture was separated and the aqueous layer was extracted with EtOAc (20 mL \times 3). The combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure. The organic residue was purified by silica gel chromatography (hexane:EtOAc = 4:1) to afford the desired adduct **A22-Phe** as a diastereomeric mixture (ca. 2:1).

A22-Phe

tert-Butyl (4*S*,5*R*)-4-benzyl-5-((phenylsulfonyl)nitromethyl)oxazolidine-3-carboxylate; 57% (281 mg); colorless oil; *The major isomer of A22-Phe*: ¹H NMR (600 MHz, 40 °C) δ 1.44 (s, 9H), 2.89 (dd, 1H, *J* = 7.2, 13.5), 3.07 (br s, 1H), 4.66 (d, 1H, *J* = 9.3), 4.70 (br s, 1H), 4.82 (t, 1H, *J* = 7.2), 5.03 (br s, 1H), 5.40 (d, 1H, *J* = 9.3, HC(NO₂)SO₂Ph), 7.05-7.93 (m, 10H); ¹³C NMR δ 28.3, 37.9, 59.1, 78.3, 78.8, 81.7, 101.2 (HC(NO₂)SO₂Ph), 127.2-136.3 (the peaks of the aryl carbons), 152.1; IR (film): 1345, 1399, 1566, 1703 cm⁻¹; HRMS (CI) calcd for C₂₂H₂₇N₂O₇S 463.1539 ([M+H]⁺), found 463.1541.

A22-Leu

tert-Butyl (4*S*,5*R*)-4-isobutyl-5-((phenylsulfonyl)nitromethyl)oxazolidine-3-carboxylate; 67% (607 mg); colorless oil; *The major isomer of A22-Leu*: ¹H NMR (600 MHz, 40 °C) δ 1.02 (d, 3H, *J* = 6.6), 1.05 (d, 3H, *J* = 6.6), 1.47-1.58 (m, 2H), 1.51 (s, 9H), 1.72-1.79 (m, 1H), 4.67 (d, 1H, *J* = 9.6), 4.71 (d,

1H, $J = 4.2$), 4.75 (t, 1H, $J = 7.2$), 5.13 (br s, 1H), 5.36 (d, 1H, $J = 9.6$, $\underline{H}C(NO_2)SO_2Ph$), 7.61-8.00 (m, 5H); ^{13}C NMR δ 22.7, 25.0, 28.4, 41.7, 56.4, 77.9, 78.3, 81.7, 101.3 ($\underline{H}C(NO_2)SO_2Ph$), 129.6-136.1 (the peaks of the aryl carbons), 152.9; IR (film): 1345, 1399, 1566, 1706 cm^{-1} ; HRMS (CI) calcd for $C_{19}H_{29}N_2O_7S$ 429.1695 ($[M+H]^+$), found 429.1696.

Oxidation procedure of A22-Phe for methyl ester A23-Phe

To a diastereomeric mixture **A22-Phe** (220 mg, 0.48 mmol) in THF (3 mL) was added methanol (3 mL) and DBU (0.22 mL, 1.43 mmol) at room temperature. The reaction mixture was cooled to $-78\text{ }^{\circ}C$ and ozone was bubbled through over 30 min. After the starting material disappeared, the reaction mixture was quenched with acetic acid. The organic solvents were removed under reduced pressure after the reaction mixture was warmed up to room temperature. Then the resulting residue was partitioned between EtOAc (20 mL) and an aqueous saturated solution of NH_4Cl (20 mL). The aqueous layer was extracted with EtOAc (20 mL \times 2), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The organic residue was purified by silica gel chromatography (hexane:EtOAc = 8:1) to afford the desired ester **A23-Phe**.

A23-Phe

3-*tert*-Butyl 5-methyl (4*S*,5*R*)-4-benzyloxazolidine-3,5-dicarboxylate; 85% (130 mg); $[\alpha]_D^{13} = -25.6$ ($c = 0.76$, $CHCl_3$); 1H NMR (50 $^{\circ}C$) δ 1.44 (s, 9H), 2.88 (dd, 1H, $J = 8.0, 13.4$), 3.07 (br d, 1H, $J = 13.4$), 3.68 (s, 3H), 4.37 (br s, 1H), 4.40 (s, 1H), 4.73 (s, 1H), 5.19 (br s, 1H), 7.19-7.30 (m, 5H); ^{13}C NMR δ 28.4, 38.5, 52.4, 60.1, 78.5, 79.5, 80.9, 126.9, 128.7, 129.6, 136.9, 152.5, 171.0; IR (film): 1165, 1700, 1752 cm^{-1} ; HRMS (CI) calcd for $C_{17}H_{24}NO_5$

322.1654 ($[M+H]^+$), found 322.1654.

General one-pot procedure for methyl ester **A23-Phe** from **A2-Phe**

To α -amino aldehyde **A2-Phe** (R = Bn, 82 mg, 0.29 mmol) in THF (0.2 mL) was added phenylsulfonylnitromethane (65 mg, 0.32 mmol) and DMAP (54 mg, 0.44 mmol). The reaction mixture was stirred at room temperature for 2 days with vigorous stirring until the starting material **A2-Phe** disappeared. The mixture was diluted with THF (3 mL) and methanol (3 mL), to which was added DBU (0.13 mL, 0.88 mmol) at room temperature. Then, the reaction mixture was cooled to -78 °C, and ozone was bubbled through over 30 min. After quenching the reaction with acetic acid (less than 1 mL), the resulting mixture was warmed up to room temperature. After removing the solvent under reduced pressure, the residue was partitioned between EtOAc (10 mL) and an aqueous saturated solution of NH_4Cl (20 mL). The aqueous layer was extracted with EtOAc (20 mL \times 3), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The organic residue was purified by silica gel chromatography (hexane:EtOAc = 8:1) to afford the desired ester **A23-Phe** (61 mg, 0.21 mmol, 72%) as a colorless oil.

A23-Leu

3-*tert*-Butyl 5-methyl (4*S*,5*R*)-4-isobutyloxazoline-3,5-dicarboxylate; 69% (54 mg); colorless oil; $[\alpha]_D^{16} = -6.7$ ($c = 0.34$, $CHCl_3$); 1H NMR (50 °C) δ 0.99 (d, 3H, $J = 6.8$), 1.01 (d, 3H, $J = 6.4$), 1.43-1.48 (m, 1H), 1.48 (s, 9H), 1.58-1.65 (m, 1H), 1.68-1.73 (m, 1H), 3.77 (s, 3H), 4.24 (t, 1H, $J = 6.6$), 4.34 (s, 1H), 4.82 (d, 1H, $J = 3.6$), 5.28 (d, 1H, $J = 3.6$); ^{13}C NMR δ 22.2, 23.0, 25.3, 28.4, 42.3, 52.4, 57.9, 79.1, 79.1, 80.8, 152.9, 171.5; IR (film): 1169, 1702,

1755 cm^{-1} ; HRMS (CI) calcd for $\text{C}_{14}\text{H}_{26}\text{NO}_5$ 288.1811 ($[\text{M}+\text{H}]^+$), found 288.1808.

A23-Val

tert-Butyl 5-methyl (4*S*,5*R*)-4-isopropylloxazolidine-3,5-dicarboxylate; 65% (109 mg), colorless oil; $[\alpha]_{\text{D}}^{17} = -10.7$ ($c = 1.17$, CHCl_3); ^1H NMR (50 $^\circ\text{C}$) δ 0.89 (d, 3H, $J = 6.4$), 0.90 (d, 3H, $J = 6.4$), 1.39 (s, 9H), 1.89-1.98 (m, 1H), 3.67 (s, 3H), 3.89 (d, 1H, $J = 5.2$), 4.36 (s, 1H), 4.72 (d, 1H, $J = 3.2$), 5.19 (br s, 1H); ^{13}C NMR δ 18.0, 18.8, 28.3, 31.0, 52.3, 64.6, 76.8, 80.0, 80.6, 153.3, 171.8; IR (film): 1173, 1711, 1757 cm^{-1} ; HRMS (CI) calcd for $\text{C}_{13}\text{H}_{24}\text{NO}_5$ 274.1654 ($[\text{M}+\text{H}]^+$), found 274.1658.

A23-Leu

3-*tert*-Butyl 5-methyl (4*S*,5*R*)-4-methylloxazolidine-3,5-dicarboxylate; 67% (81 mg), colorless oil; $[\alpha]_{\text{D}}^{15} = -5.9$ ($c = 0.44$, CHCl_3); ^1H NMR (50 $^\circ\text{C}$) δ 1.37 (d, 3H, $J = 6.4$), 1.46 (s, 9H), 3.77 (s, 3H), 4.14 (br s, 1H), 4.24 (d, 1H, $J = 3.2$), 4.84 (d, 1H, $J = 3.8$), 5.20 (d, 1H, $J = 3.8$); ^{13}C NMR δ 19.3, 28.5, 52.6, 55.0, 79.2, 80.8, 81.9, 152.6, 170.9; IR (film): 1175, 1707, 1756 cm^{-1} ; HRMS (CI) calcd for $\text{C}_{11}\text{H}_{20}\text{NO}_5$ 246.1341 ($[\text{M}+\text{H}]^+$), found 246.1345.

A23-Ser

3-*tert*-Butyl 5-methyl (4*S*,5*R*)-4-((*tert*-butyldimethylsilyloxy)methyl)oxazolidine-3,5-dicarboxylate; 79% (93 mg), colorless oil; $[\alpha]_{\text{D}}^{15} = -10.5$ ($c = 0.71$, CHCl_3); ^1H NMR (50 $^\circ\text{C}$) δ 0.06 (s, 3H), 0.07 (s, 3H), 0.90 (s, 9H), 1.46 (s, 9H), 3.72-3.81 (m, 2H), 3.77 (s, 3H), 4.07 (br s, 1H), 4.71 (d, 1H, $J = 3.6$), 4.79 (d, 1H, $J = 3.2$), 5.19 (br s, 1H); ^{13}C NMR δ -5.3, 18.2, 25.9, 28.5, 52.5, 60.2, 61.7, 77.9, 80.1, 80.9, 152.5, 171.3; IR (film): 839, 1175, 1709, 1752 cm^{-1} ; HRMS (CI) calcd for $\text{C}_{17}\text{H}_{34}\text{NO}_6\text{Si}$

376.2155 ($[M+H]^+$), found 376.2159.

Stepwise procedure for methyl ester **A23**

General procedure for nitromethyloxazolidine **A24-Phe** or **A24-Leu**

To conjugate adduct **A2-Phe** (R = Bn, 67 mg, 0.15 mmol) in toluene (2 mL) was added *N*-benzylnicotinamide (BNAH, 93 mg, 0.44 mmol) and AIBN (5 mg, 0.03 mmol). The reaction mixture was reacted at 60 °C for 1 h in a microwave (MW) reactor (15 W). Then, the mixture was diluted with EtOAc (2 mL), to which was added H₂O (5 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (10 mL \times 3). The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure. The organic residue was purified by silica gel chromatography (hexane:EtOAc = 8:1) to afford nitromethyloxazolidine **A24-Phe**.

A24-Phe

tert-Butyl (4*S*,5*R*)-4-benzyl-5-(nitromethyl)oxazolidine-3-carboxylate; 62% (29 mg); colorless oil; $[\alpha]_D^{15} = -5.7$ ($c = 2.53$, CHCl₃); ¹H NMR (50 °C) δ 1.51 (s, 9H), 2.82 (dd, 1H, $J = 9.2, 12.0$), 3.26 (br d, 1H, $J = 12.0$), 3.95-4.01 (m, 2H), 4.32 (dd, 1H, $J = 8.4, 12.8$), 4.58-4.63 (m, 1H), 4.69 (d, 1H, $J = 4.0$), 5.17 (br s, 1H), 7.23-7.37 (m, 5H); ¹³C NMR δ 28.4, 38.7, 59.2, 76.5, 78.6, 78.8, 81.4, 127.4, 129.1, 129.4, 136.4, 152.8; IR (film): 1370, 1557, 1679 cm⁻¹; HRMS (CI) calcd for C₁₆H₂₃N₂O₅ 323.1607 ($[M+H]^+$), found 323.1611.

A24-Leu

tert-Butyl (4*S*,5*R*)-4-isobutyl-5-(nitromethyl)oxazolidine-3-carboxylate; 72% (50 mg); colorless oil; $[\alpha]_D^{14} = -8.2$ ($c = 0.26$, CHCl₃); ¹H NMR (50 °C) δ 0.98 (d, 3H, $J = 6.4$), 1.02 (d, 3H, $J = 6.4$), 1.43-1.51 (m, 1H), 1.51 (s, 9H), 1.61-

1.71 (m, 2H), 3.98 (t, 1H, $J = 6.4$), 4.39-4.51 (m, 3H), 4.74 (d, 1H, $J = 4.8$), 5.20 (d, 1H, $J = 4.8$); ^{13}C NMR δ 22.5, 23.0, 25.2, 28.5, 42.2, 56.9, 76.8, 78.2, 79.1, 81.3, 153.2; IR (film): 1372, 1559, 1704 cm^{-1} ; HRMS (CI) calcd for $\text{C}_{13}\text{H}_{25}\text{N}_2\text{O}_5$ 289.1763 ($[\text{M}+\text{H}]^+$), found 289.1760.

Two-step procedure for A23-Phe from A24-Phe

Nitromethyloxazolidine **A24-Phe** ($\text{R} = \text{Bn}$, 73 mg, 0.23 mmol) in *t*-BuOH (2 mL) was stirred in an aqueous solution of 0.5 M KOH buffered with 1.25 M of K_2HPO_4 (1.5 mL) at room temperature for 5 min. To the reaction mixture an aqueous solution of 0.5 M KMnO_4 (0.90 mL) was slowly added at 0 °C and it was stirred at room temperature for 1 h. The reaction was quenched with an aqueous saturated solution of Na_2SO_3 (5 mL) at 0 °C, and the reaction mixture was acidified with an aqueous solution of 1 M HCl (6 mL). The resulting mixture was separated, and the aqueous layer was extracted with EtOAc (10 mL \times 3). The combined organic layers were dried over MgSO_4 , filtered and concentrated under reduced pressure to afford the corresponding acid intermediate **A25-Phe**. To crude acid **A25-Phe** in DMF (2 mL) was added K_2CO_3 (94 mg, 0.68 mmol) and MeI (0.03 mL, 0.45 mmol). After 1 h at room temperature, the mixture was poured into cold water (10 mL). The resulting mixture was extracted with diethyl ether (10 mL \times 3). The combined organic layers were dried over MgSO_4 , filtered and concentrated under reduced pressure. The organic residue was purified by silica gel chromatography (hexane:EtOAc = 8:1) to afford the desired ester **A23-Phe** (51 mg, 70%) as a colorless oil.

General procedure for β -amino- α -hydroxy acids, AHPBA·HCl or AHMHA·HCl, from A23-Phe and A23-Leu

Methyl ester **A23-Phe** (R = Bn, 152 mg, 0.47 mmol) in an aqueous solution of 3 M HCl (8 mL) was heated at 80 °C for 8 h. After cooling back to room temperature, the resulting mixture was washed with CH₂Cl₂ (20 mL × 2) and EtOAc (20 mL × 2). The aqueous layer was concentrated under reduced pressure to give **AHPBA·HCl** salt.

AHPBA·HCl

(2*R*,3*S*)-3-Amino-2-hydroxy-4-phenylbutanoic acid hydrochloride; 92% (85 mg); white solid; mp. 206-208 °C (lit.^{27a}; 209-210 °C); [α]_D²⁶ = -28.8 (*c* 1.68, 1 M HCl); (lit.^{27b} [α]_D = +29.3 (*c* 0.43, 1 M HCl)); ¹H NMR (DMSO-*d*₆) δ 2.88 (dd, 1H, *J* = 9.6, 13.4), 3.03 (dd, 1H, *J* = 5.2, 13.4), 3.58-3.60 (m, 1H), 3.89 (d, 1H, *J* = 2.4), 7.27-7.36 (m, 5H), 8.18 (br s, 1H); ¹³C NMR δ 35.1, 53.9, 67.4, 126.9, 128.6, 129.3, 136.2, 172.7; IR (film); 1072, 1730, 2931 3040 cm⁻¹; HRMS (CI) calcd for C₁₀H₁₄NO₃ 196.0974 ([M+H]⁺), found 196.0971.

AHMHA·HCl

(2*R*,3*S*)-3-Amino-2-hydroxy-5-methylhexanoic acid hydrochloride; 88% (93 mg); white solid; mp. 208-210 °C (lit.^{29a} 188-189 °C, lit.^{29b} 228-229 °C) ¹H NMR (MeOH-*d*₄) δ 0.98 (d, 3H, *J* = 6.4), 1.00 (d, 3H, *J* = 6.4), 1.47-1.54 (m, 1H), 1.64-1.79 (m, 2H), 3.56 (dt, 1H, *J* = 3.2, 7.0), 4.23 (d, 1H, *J* = 3.2); ¹³C NMR δ 22.5, 22.7, 25.4, 39.8, 52.8, 70.4, 174.4; IR (film): 1101, 1657, 2960, 3367 cm⁻¹; HRMS (CI) calcd for C₇H₁₆NO₃ 162.1130 ([M+H]⁺), found 162.1131. The free amino acid form of (2*R*,3*S*)-AHMHA was obtained in a quantitative yield by treating (2*R*,3*S*)-**AHMHA·HCl** with a Dowex resin (50WX8); [α]_D²⁸ = +25.0 (*c* 1.28, AcOH), (lit.^{29b} [α]_D²⁵ = +25.2 (*c* 0.6, AcOH)).

Peptide synthesis from methyl ester *ent*-A23-Phe

Preparation of *ent*-A25-Phe

To methyl ester **ent-A23-Phe** (R = Bn, 283 mg, 0.88 mmol) in THF (2 mL) was added an aqueous solution of 2 M NaOH (6 mL), and the reaction mixture was stirred at room temperature over 2 h. After the reaction mixture was diluted with EtOAc (10 mL), the pH was adjusted to ca. 1 with an aqueous solution of 1 M HCl. The aqueous phase was extracted with EtOAc (20 mL \times 2). The combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure to afford the corresponding acid **ent-A25-Phe**

ent-A25-Phe

3-(*tert*-Butoxycarbonyl)-(4*R*,5*S*)-4-benzyloxazolidine-5-carboxylic acid; 92% (249 mg); $[\alpha]_{\text{D}}^{14} = +17.0$ ($c = 0.92$, CHCl₃); ¹H NMR δ 1.43 (s, 9H), 2.90 (dd, 1H, $J = 8.2, 13.4$), 3.07 (br s, 1H), 4.43 (br s, 1H), 4.43 (s, 1H), 4.74 (br s, 1H), 5.18 (br s, 1H), 7.19-7.33 (m, 5H), 9.82 (s, 1H); ¹³C NMR δ 28.4, 38.5, 60.1, 78.2, 79.5, 81.4, 127.0, 128.8, 129.6, 136.6, 152.7, 175.1; IR (film): 1704, 1750, 2978 cm⁻¹; HRMS (CI) calcd for C₁₆H₂₂NO₅ 308.1498 ([M+H]⁺), found 308.1495.

General procedure for dipeptides A28-Phe

To crude **ent-25A-Phe** (76 mg, 0.23 mmol) in THF (25 mL) at 0 °C was added *N*-methylmorpholine (27 μ L, 0.25 mmol) and isobutyl chloroformate (32 μ L, 0.25 mmol). After 5 min, L-leucine methyl ester (47 mg, 0.26 mmol) was added to the reaction mixture, and then the mixture was stirred at room temperature for 5 h. After the removal of the solvents, the residue was partitioned between H₂O (20 mL) and EtOAc (20 mL). The aqueous phase

was extracted with EtOAc (20 mL \times 2), and the combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure. The organic residue was purified by silica gel chromatography (hexane:EtOAc = 8:1) to afford the desired ester of dipeptide **A28-Phe**.

A28-Phe

[3-(*tert*-Butoxycarbonyl-(4*R*,5*S*)-4-benzyloxazolidine-5-carboxylic acid)]-L-leucine methyl ester; 92% (94 mg); colorless oil; $[\alpha]_{\text{D}}^{25} = -10.7$ ($c = 0.96$, CHCl₃); ¹H NMR (50 °C) δ 0.94 (d, 6H, $J = 6.0$), 1.40 (s, 9H), 1.52-1.63 (m, 2H), 1.64-1.70 (m, 1H), 2.99 (dd, 1H, $J = 5.8, 13.4$), 3.08 (dd, 1H, $J = 5.8, 13.4$), 3.74 (s, 3H), 4.34 (d, 1H, $J = 3.2$), 4.49-4.53 (m, 1H), 4.65-4.60 (m, 1H), 6.78 (d, 1H, $J = 8.0$), 7.22-7.33 (m, 5H); ¹³C NMR δ 21.8, 22.8, 24.9, 28.1, 38.8, 41.4, 50.2, 52.3, 60.0, 78.7, 80.5, 80.7, 126.7, 128.5, 129.8, 136.8, 152.4, 169.9, 172.7; IR (film): 1156, 1698, 1742, 3335 cm⁻¹; HRMS (CI) calcd for C₂₃H₃₅N₂O₆ 435.2495 ([M+H]⁺), found 435.2491.

A28-Phe' for AHPBA-L-Val

[3-(*tert*-Butoxycarbonyl-(4*R*,5*S*)-4-benzyloxazolidine-5-carboxylic acid)]-L-valine methyl ester; 70% (198 mg); colorless oil; $[\alpha]_{\text{D}}^{25} = -0.8$ ($c = 0.54$, CHCl₃); ¹H NMR (50 °C) δ 0.89 (d, 3H, $J = 6.0$), 0.93 (d, 3H, $J = 6.8$), 1.38 (s, 9H), 2.14-2.22 (m, 1H), 3.00 (dd, 1H, $J = 6.2, 13.8$), 3.08 (dd, 1H, $J = 6.2, 13.8$), 3.76 (s, 3H), 4.35 (d, 1H, $J = 3.2$), 4.53-4.58 (br s, 1H), 5.28 (br s, 1H), 6.9 (d, 1H, $J = 8.0$), 7.23-7.33 (m, 5H); ¹³C NMR δ 21.8, 22.8, 24.9, 28.1, 38.8, 41.4, 50.2, 52.3, 60.0, 78.7, 80.5, 80.7, 126.7, 128.5, 129.8, 136.8, 152.4, 169.9, 172.7; IR (film): 1162, 1696, 1741, 3354 cm⁻¹; HRMS (CI) calcd for C₂₂H₃₃N₂O₆ 421.2339 ([M+H]⁺), found 421.2337.

A28-Leu

[3-*tert*-Butyl 5-methyl (4*R*,5*S*)-4-isobutyloxazoline-5-carboxylic acid]-L-leucine methyl ester 74% (370 mg); colorless oil; ^1H NMR δ 0.94 (dd, 3H, J = 2.6, 6.2), 1.00 (t, 3H, J = 6.4), 1.47 (s, 9H), 1.54-1.63 (m, 3H), 1.66-1.77 (m, 2H), 3.76 (s, 3H), 4.22 (d, 1H, J = 2.8), 4.38 (dt, 1H, J = 2.5, 7.3), 4.61-4.4.77 (m, 1H), 4.77 (d, 1H, J = 4.8), 5.31 (br d, 1H, J = 3.2), 6.86 (d, 1H, J = 8.4); ^{13}C NMR δ 21.9, 22.4, 22.8, 24.9, 25.0, 28.3, 41.4, 42.7, 50.3, 52.3, 57.9, 78.2, 80.9, 81.7, 152.9, 170.4, 172.9.

A28-Val

[3-*tert*-Butyl 5-methyl (4*R*,5*S*)-4-isopropylloxazoline-5-carboxylic acid]-L-leucine methyl ester 76% (264 mg); colorless oil; ^1H NMR δ 0.92 (dd, 3H, J = 1.6, 6.4), 0.99 (t, 3H, J = 7.0), 1.45 (s, 9H), 1.54-1.61 (m, 2H), 1.63-1.69 (m, 1H), 1.94-1.99 (m, 1H), 3.74 (s, 3H), 4.09-4.11 (m, 1H), 4.34 (d, 1H, J = 2.8), 4.59-4.73 (m, 1H), 4.74 (d, 1H, J = 4.8), 5.32 (br s, 1H), 6.91 (d, 1H, J = 8.4); ^{13}C NMR δ 18.5, 18.8, 21.8, 22.8, 24.9, 28.2, 28.3, 31.4, 41.4, 50.3, 52.3, 64.8, 79.2, 79.9, 80.9, 153.3, 170.9, 172.9. ; HRMS (FAB) calcd for $\text{C}_{19}\text{H}_{35}\text{N}_2\text{O}_6$ 387.2495 ($[\text{M}+\text{H}]^+$), found 387.2496.

A29

(2*S*,3*R*)-3-Amino-2-hydroxy-5-methylhexanamido)-4-methylpentanoic acid; 95% (235 mg); white powder; ^1H NMR δ 0.85-0.93 (m, 12H), 1.36-1.50 (m, 2H), 1.56-1.60 (m, 1H), 1.63-1.69 (m, 2H), 1.70-1.78 (m, 1H), 4.09 (d, 1H, 4.8), 4.30 (dt, 1H, J = 4.8, 8.4), 6.44 (br s, 1H), 7.77 (br s, 2H), 8.12 (d, 1H, J = 7.6); ^{13}C NMR δ 21.2, 21.4, 22.4, 22.4, 23.1, 24.1, 37.3, 50.0, 51.3, 69.7, 170.7, 173.1.; HRMS (FAB) calcd for $\text{C}_{13}\text{H}_{27}\text{N}_2\text{O}_4$ 275.1971 ($[\text{M}+\text{H}]^+$), found 275.1977.

A30

(2*S*,3*R*)-3-Amino-2-hydroxy-4-methylpantanamido)-4-methylpentanoic acid; quant. (223 mg); pale yellow powder; ^1H NMR δ 0.91 (m, 12H), 1.38-1.47 (m, 2H), 1.56-1.78 (m, 2H), 3.25 (d, 1H, $J = 6.8$), 4.09 (d, 1H, $J = 6.8$), 4.30 (qt, 1H, $J = 4.4$), 6.46 (br s, 1H), 7.77 (s, 2H), 8.15 (s, 1H); ^{13}C NMR δ 21.2, 21.4, 22.4, 224, 23.1, 24.1, 37.4, 50.0, 51.3, 69.8, 170.8 173.1.

Procedure for A31

To a solution of *ent*-**A23-Ser** (297 mg, 0.791 mmol) in THF was added TBAFH₂O (213 mg, 0.815 mmol). The reaction mixture was stirred at for 1 h at room temperature and then partitioned between the saturated NH₄Cl aqueous solution (10 mL \times 2) and EtOAc (20 mL \times 2). The comined organic layers were dried with MgSO₄, filtered and concentrated under reduced pressure.

To the obtained crude of the desilylated product in acetone (3 mL) and an aqueous solution of 5% NaHCO₃ (10 mL) at 0 °C was added KBr (18 mg), TEMPO (185 mg) and then an aqueous solution of 12.5% NaOCl (3.164 mL). The resulting mixture was stirred for 3 h at room temperature and then partitioned between H₂O (20 mL \times 2) and Et₂O (20 mL \times 2). The combined aqueous layers were acidified with an aqueous solution of 2 M HCl to pH 4~5 and then the resulting mixture was partitioned between brine (20 mL \times 2) and EtOAc (20 mL \times 2). The combined organic layers were dried with MgSO₄, filtered, and concentrated under reduced pressure.

To the obtained crude in diethyl ether (6 mL) and MeOH (2 mL), 2 M THF solution of TMSCHN₂ was added, and then the reaction mixture was stirred at room temperature for 2 h. After the reaction was completed, the reaction

mixture was condensed under reduced pressure. The residue was purified with SiO₂ column chromatography (4:1 Hex/EtOAc) to give **A31** as colorless oil.

A31

(4*S*,5*S*)-3-*tert*-Butyl 4,5-dimethyl oxazolidine-3,4,5-tricarboxylate; 89% (204 mg); colorless oil; ¹H NMR δ 1.48 (s, 9H), 3.84 (s, 3H), 3.85 (s, 3H), 4.61-4.75 (br d, 1H), 4.80 (d, 1H, *J* = 2.0), 5.02-5.10 (br d, 1H), 5.23 (s, 1H); ¹³C NMR δ 28.2, 52.8, 59.5, 60.2, 78.3, 79.8, 81.6, 151.7, 152.3, 169.4, 169.6.; HRMS (CI) calcd for C₁₂H₂₀NO₇ 290.1240 ([M+H]⁺), found 290.1237.

Procedure for A32

To a solution of **A31** (93 mg, 0.321 mmol) in anhydrous CH₂Cl₂ (2 mL), TFA (1 mL) was added then stirred at room temperature overnight. After the removal of the organic solvents, the residue was partitioned between H₂O (10 mL × 2) and EtOAc (10 mL × 2). The combined aqueous layers were then condensed under reduced pressure.

To the crude (88 mg, 0.321 mmol) in anhydrous MeOH (3 mL), Boc₂O (0.103 mL, 0.482 mmol) and Na₂CO₃ (51 mg, 0.482) were added, and then stirred under N₂. After the reaction was completed, the reaction mixture was condensed under reduced pressure. Then the residue in EtOAc (10 mL) was acidified with an aqueous solution of 2 M HCl to pH 1 and then the resulting mixture was partitioned between brine (20 mL × 2) and EtOAc (20 mL × 2). The combined organic layers were dried with MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified with SiO₂ column chromatography (4:1 Hex/EtOAc) to give **A32** as colorless oil.

A32

(2*S*,2*S*)-Dimethyl 2-((*tert*-butoxycarbonyl)amino)-3-hydroxysuccinate; 43%

(37 mg); colorless oil; ^1H NMR δ 1.46 (s, 9H), 3.17 (d, 1H, $J = 5.9$), 3.84 (s, 3H), 3.85 (s, 3H), 4.72 (dd, 1H, $J = 1.8, 5.9$), 4.81 (d, 1H, $J = 9.0$), 5.29 (d, 1H, $J = 9.0$); ^{13}C NMR δ 28.4, 52.9, 53.2, 56.1, 71.0, 80.4, 155.3, 166.9, 172.1; HRMS (CI) calcd for $\text{C}_{12}\text{H}_{20}\text{NO}_7$ 290.1240 ($[\text{M}+\text{H}]^+$), found 290.1237.

Procedure for A33

To a solution of **A32** (37 mg, 0.13 mmol) in DMF, BnBr (0.079 mL, 0.67), Ag_2O (186 mg, 0.80 mmol), and TBAI (296 mg, 0.80 mmol) were added. Then the mixture was reacted in the dark overnight. The reaction mixture was filtered through Celite, washing through with ether (30 mL). The organic solution was washed with water (30 mL \times 3). The organic layers were dried with MgSO_4 and concentrated under reduced pressure. The residue was purified with SiO_2 column chromatography (2:1 Hex/EtOAc) to give **A33** as colorless oil.

A33

(2*S*,2*S*)-Dimethyl 2-(benzyloxy)-3-((*tert*-butoxycarbonyl)amino)succinate; 55% (27 mg); colorless oil; ^1H NMR δ 1.45 (s, 9H), 3.65 (s, 3H), 3.77 (s, 3H), 4.39 (d, 1H, $J = 12.0$), 4.51 (d, 1H, $J = 2.1$), 4.85 (dd, 1H, $J = 2.1, 9.9$), 4.84 (d, 1H, $J = 12.0$), 5.39 (br d, 1H, $J = 9.9$); ^{13}C NMR δ 28.2, 52.4, 52.6, 72.8, 80.2, 128.1, 128.2, 128.3, 136.6, 155.5, 169.7, 169.7.

Procedure for L-TBOA

To the solution of A33 in THF, 2 M NaOH aqueous solution was added, and stirred at room temperature for 2 h. After the reaction was completed, the residue in EtOAc (10 mL) was acidified with an aqueous solution of 2 M HCl to pH 1 and then the resulting mixture was partitioned between brine (20 mL \times 2) and EtOAc (20 mL \times 2). The combined organic layers were dried with

MgSO₄, filtered, and concentrated under reduced pressure.

To the solution of crude in anhydrous CH₂Cl₂ (2 mL), TFA (0.5 mL) was added and stirred at room temperature overnight. After the removal of the organic solvents, the residue was partitioned between H₂O (10 mL × 2) and EtOAc (10 mL × 2). The combined aqueous layers were then condensed under reduced pressure to obtain L-TBOA as colorless oil.

L-TBOA

(2*S*,2*S*)-2-Amino-3-(benzyloxy)succinic acid; 93% (23 mg); colorless oil; ¹H NMR (DCI) δ 4.22 (d, 1H, *J* = 2.4), 4.27 (d, 1H, *J* = 11.6), 4.36 (d, 1H, *J* = 2.4), 4.52 (d, 1H, *J* = 11.6), 7.05-7.11 (m, 5H); ¹³C NMR δ 54.3, 73.4, 73.9, 128.6, 128.7, 128.8, 135.7, 168.4, 171.5; HRMS (CI) calcd for C₁₁H₁₄NO₅ 240.0872 ([M+H]⁺), found 240.0873.

General procedure for dipeptides *ent*-A34

To *ent*-A23-Val (183 mg, 0.67 mmol) in THF (6 mL) at 0 °C was added 2 N NaOH (6 mL). After the reaction mixture was stirred at room temperature for 1 h, it was acidified with 2 N HCl to pH 1 at 0 °C. The reaction mixture was then partitioned between EtOAc (20 mL) and brine (5 mL). The aqueous phase was extracted with EtOAc (20 mL × 2), and the combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure.

To the obtained crude in THF at 0 °C, HOBt (109 mg, 0.804 mmol) and L-Val-O'Bu (154 mg, 0.737 mmol) were added. EDC (154 mg, 0.804 mmol), and *N,N*-diisopropylethylamine (0.282 mL, 2.01 mmol) were added to the reaction mixture. The mixture was stirred at room temperature overnight. After the removal of the solvents, the residue was partitioned between the

saturated aqueous solution of NH_4Cl (20 mL) and EtOAc (20 mL). The aqueous phase was extracted with EtOAc (20 mL \times 2), and the combined organic layers were dried over MgSO_4 , filtered and concentrated under reduced pressure. The organic residue was purified by silica gel chromatography (hexane:EtOAc = 8:1) to afford the desired ester of dipeptide **ent-A34**.

ent-A34

[3-*tert*-Butyl 5-methyl (4*R*,5*S*)-4-isopropylloxazoline-5-carboxylic acid]-L-valine *tert*-butyl ester; 54% (149 mg); colorless oil; ^1H NMR δ 0.87 (d, 3H, J = 6.8), 0.92 (d, 3H, J = 7.2), 1.01 (d, 3H, J = 6.4), 1.04 (d, 3H, J = 6.8), 1.46 (s, 9H), 1.97 (br s, 1H), 2.15-2.23 (m, 1H), 4.35 (d, 1H, J = 2), 4.46 (dd, 1H, J = 4.6, 8.7), 4.78 (d, 1H, J = 4.6), 5.42 (br s, 1H), 7.04 (d, 1H, 8.7); ^{13}C NMR δ 17.4, 17.6, 18.7, 18.8, 18.9, 18.9, 19.0, 19.2, 28.1, 31.5, 56.8, 65.3, 79.3, 80.7, 82.2, 153.4, 170.6, 171.0; HRMS (FAB) calcd for $\text{C}_{21}\text{H}_{39}\text{N}_2\text{O}_6$ 415.2808 ($[\text{M}+\text{H}]^+$), found 415.2792.

A35

(2*S*,3*R*)-3-Amino-2-hydroxy-4-methylpentanamido-3-methylbutanoic acid; quant. (133 mg); colorless oil; ^1H NMR ($\text{DMSO}-d_6$) δ 0.91 (d, 1H, J = 7.2), 0.92 (d, 1H, J = 6.8), 0.95 (d, 1H, J = 8.8), 0.97 (d, 1H, J = 9.2), 1.88-1.95 (m, 1H), 2.07-2.15 (m, 1H), 3.03 (d, 1H, J = 4.4), 4.15 (dd, 1H, J = 5.6, 8.0), 4.26 (d, 1H, J = 4.4), 7.77 (s, 2H), 8.13 (d, 1H, J = 8.0); ^{13}C NMR δ 17.8, 18.1, 18.9, 19.1, 27.5, 29.8, 57.1, 58.0, 68.2, 171.4, 172.4.

Part B.

Cu-catalyzed one-pot process for acetaminophen

Introduction

1. Acetaminophen

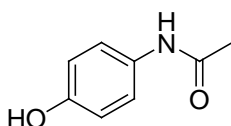


Figure B1. The structure of acetaminophen.

Acetaminophen (Fig. B1), also known as paracetamol or *N*-acetyl-*p*-aminophenol,¹ is a popular analgesic for headache or other minor pains, and it is also a major ingredient in cold and flu treatment as an antipyretic. These analgesic and antipyretic properties of acetaminophen are similar to that of non-steroidal anti-inflammatory drugs (NSAIDs), but it does not possess any anti-inflammatory activity, which is contrary to NSAIDs. When acetaminophen is applied in recommended doses, it does not induce gastrointestinal bleeding that is a typical side effect from NSAIDs although it suppresses prostaglandin production likewise NSAIDs. Due to lack of an anti-inflammatory effect, acetaminophen has not been regarded as a member of the NSAIDs family.

Acetaminophen was introduced into the pharmacological market in 1955 by McNeil laboratories as a prescribed analgesic and antipyretic drug for children

under its trade name *Tylenol Children's Elixir*. The discovery of acetaminophen was derived from acetanilide (Fig. B2), whose antipyretic effect was found by chance during the irradiation of worms in 1880s.² For many years after its discovery in 1886, acetanilide was used as an alternative to aspirin (acetyl salicylate) that showed gastrointestinal, ulcer or stomach bleeding and ringing in the ears, especially with higher doses. Acetanilide, however, could not be used as antipyretic medicine due to its high toxicity since 1948, when Julius Axelrod and Bernard Brodie discovered that acetanilide caused methemoglobinemia and ultimately caused damage to the liver and kidneys.

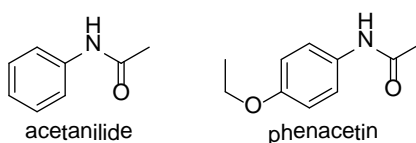
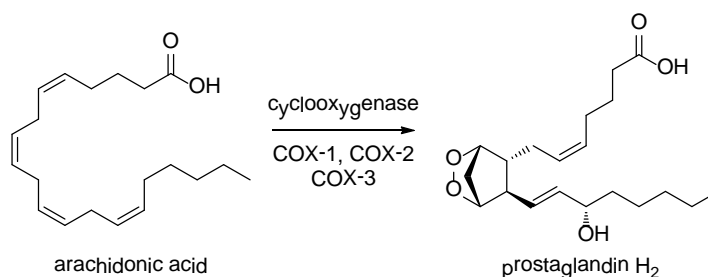


Figure B2. Structure of acetanilide and phenacetin.

In order to alleviate toxicity of acetanilide, phenacetin and acetaminophen had been synthesized as its derivatives.³ Among the derivatives, phenacetin (Fig. B2) was introduced into medical practice in 1887 earlier than acetaminophen due to the faulty conclusion on the first clinical trials of acetaminophen. In the first trials with acetaminophen, its toxicity was estimated as much as that of acetanilide, but Bernard Brodies and Julius Axelrod demonstrated that acetaminophen was the main active metabolite of acetanilide and phenacetin responsible for their analgesic and antipyretic action in 1948.⁴ Since then, acetaminophen had become a popular analgesic and antipyretic drug while phenacetin had been less treated as an analgesic

because of its side effect, which caused the analgesic nephropathy with a prolonged usage.⁵

Although acetaminophen had been widely used in medical practice for more than half the century, its mechanism of action has not been elucidated until now.⁶ The verified effects are known as being related to both the peripheral (cyclooxygenase (COX) inhibition), and central (COX, serotonergic descending neuronal pathway, L-arginine/NO pathway, cannabinoid system) antinociception process and “redox” mechanism.⁴ As like shown in Scheme B1, the COX enzymes are responsible for the conversion of arachidonic acid to prostaglandin H₂, which is an unstable molecule that is converted to numerous other pro-inflammatory compounds. In the production of prostaglandin H₂, the COX enzymes are participated as thier oxidized forms. Therefore, the anti-inflammatory agents usually block the oxidation of the COX enzyme so that the unstable molecule for pro-inflammatory compounds could not be synthesized by COX enzyme. Acetaminophen also reduces the oxidized form of the COX enzymes.



Scheme B1. Prostaglandin H₂ synthesis with COX.

Based on its relatively stable nature of acetaminophen, more than 600 different over-the-counter and prescription medicines containing it have been

distributed worldwide. According to the report by FDA in 2009, over 370 million bottles and packets (24.5 billion tablets) of acetaminophen products was sold in 2008, and it recorded \$2.6 billion in sale (Fig. B3). Furthermore, 28% of growth in overall acetaminophen market between 2004 and 2008 was reported.⁷

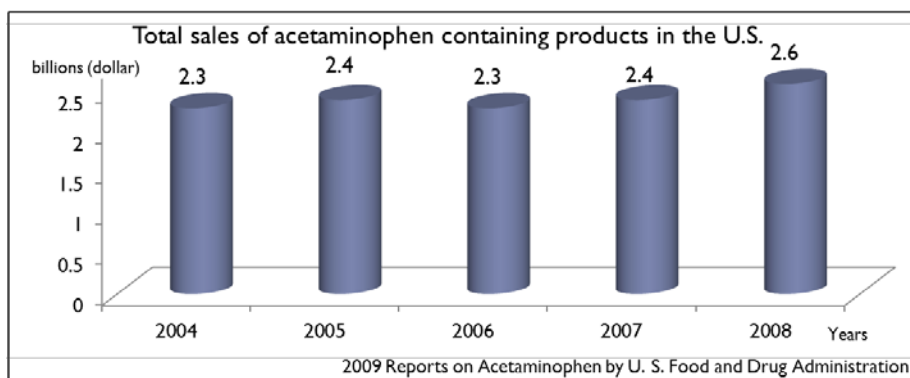


Figure B3. Total sales of acetaminophen containing products in the US.⁷

Acetaminophen has also permeated as an easily accessible analgesic in Korea. Tyrenol, one of the typical over-the-counter analgesics including acetaminophen as a main ingredient, was sold over 25 billion won in 2011 and the total sales of the other popular analgesics containing acetaminophen, such as Geworin, Panpyrin Q and Ultracet, was recorded in 77 billion won in 2011 according to the report from Korea Health Industry Development Institute (Fig. B4).⁸

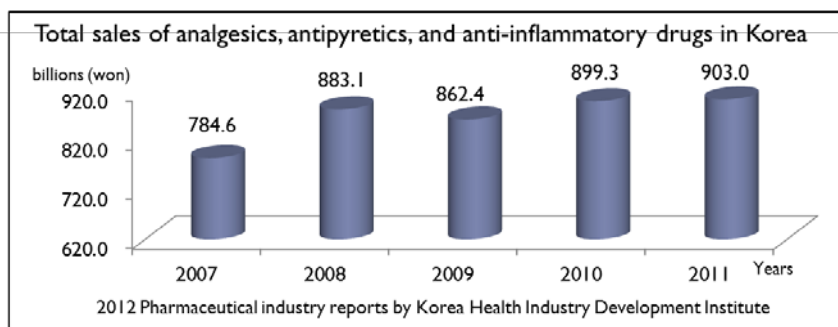
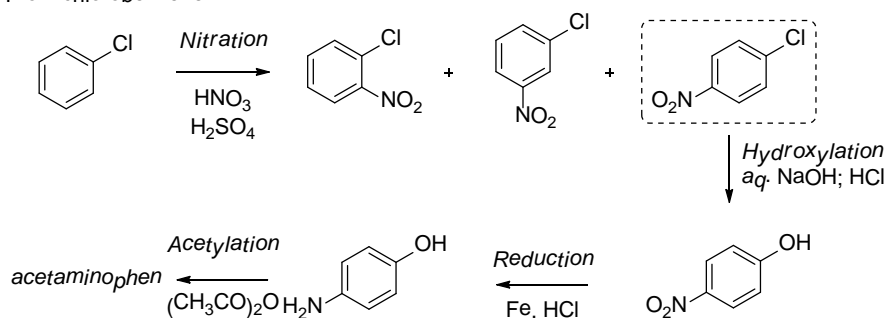


Figure B4. Total sales of analgesics & anti-inflammatory drugs in Korea.

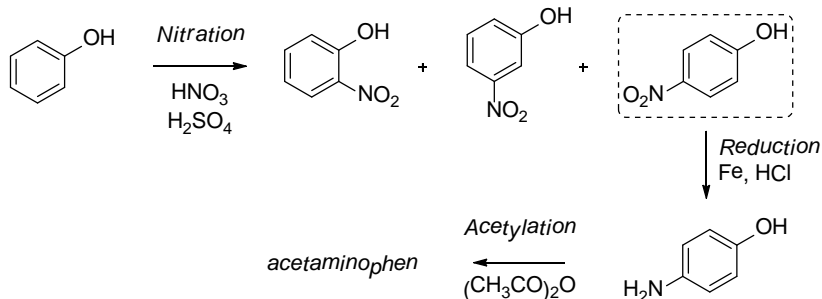
2. Conventional process for Acetaminophen

The conventional process for acetaminophen started with chlorobenzene or phenol (Scheme B2). Regardless of the starting material, the first step is nitration, which produces the mixture of *o*-, *m*-, *p*- regioisomers, and the desired *p*-regioisomer, *p*-chlorobenzene or *p*-nitrophenol, is separated before the next step. The separated *p*-chlorobenzene is hydroxylated to the *p*-nitrophenol.

(a) From chlorobenzene



(b) From phenol



Scheme B2. Conventional process for acetaminophen.

The key intermediate, *p*-aminophenol, is produced by the reduction of *p*-nitrophenol, and then the following acetylation of the intermediate completes

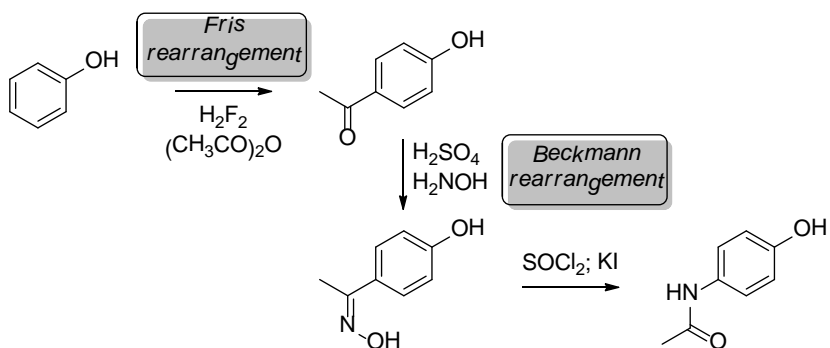
the conventional process for acetaminophen.¹⁰ Because the nitration of chlorobenzene or phenol accompanies with the undesired regioisomers that require an additional separation process for the desired *p*-nitro compounds, the conventional process has been developed for more efficient production of *p*-aminophenol with reducing the undesired regioisomers.

Two alternative routes have been reported to improve the regioselectivity on the synthesis of *p*-aminophenol. One of the alternative processes is Hoechst-Celanes process which utilizes Fries rearrangement for the preparation of acetophenone from phenol and the following Beckmann rearrangement converting the acetoxy moiety to acetamido functional group (Scheme B2 (a)).

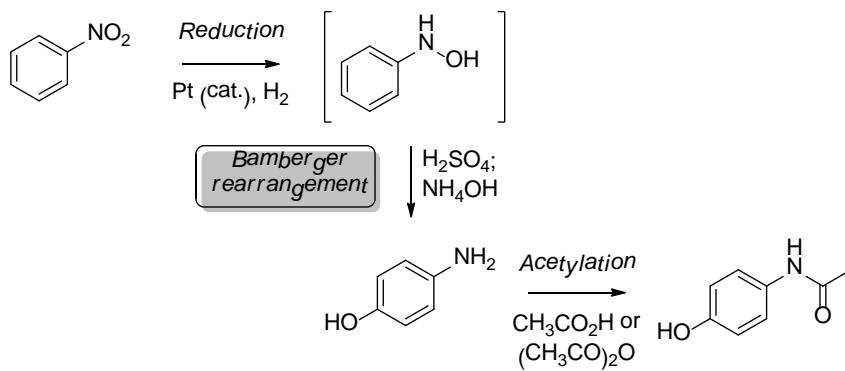
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Another alternative route starts with nitrobenzene, which is readily prepared from the nitration of benzene. Under the catalytic hydrogenation reaction conditions, nitro group is reduced to *N*-phenylhydroxylamine, and then the hydroxylamine is successfully converted to *p*-aminophenol under acidic reaction conditions via Bamberger rearrangement.¹² The acetylation of the amino group on the *p*-regioisomer affords the desired acetaminophen (Scheme B2 (b)).

(a) Hoechst-Celanese process (Fris and Beckmann rearrangement)



(b) Bamberger rearrangement



Scheme B3. Alternative processes for acetaminophen.

However, these alternative processes also reveal some drawbacks. In Hoechst-Celanese process, the used acid catalyst (H_2F_2) is too virulent to be used in large scale process although the condensation with acetic anhydride and phenol under the acid catalyst has been successful. In Bamberger rearrangement with nitrobenzene the production of aniline is a predictable defect of the process even though nitrobenzene is cheaper than the nitrophenol. Like this, the alternative processes have not completely reformed the selectivity issue in the conventional process due to their own problems. Besides the regioselectivity, furthermore, the conventional process involves other drawbacks.

The process environment of acetaminophen production on the conventional or the alternative methods could be considered as harmful or sometimes hazardous because mineral acids are used in the key reactions, even sometimes used as reaction solvents, which are applied as aqueous solution of them. The usage of mineral acids causes the erosion of the reactor, and an additional process to treat the waste acids is necessary at the end of the reaction.

Another drawback is related to the quality control. The synthesized acetaminophen has to meet the National Formulary specification in order to qualify for use in the pharmaceutical field and indeed in many non-pharmaceutical applications. However, acetaminophen from the process containing *p*-aminophenol as a key intermediate often contains numerous colored compounds or colored impurities, which are exceedingly difficult to remove unfortunately.

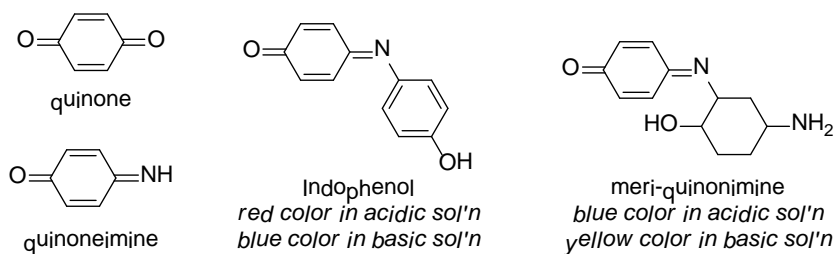


Figure B5. Possible colored impurities on the synthesis of *p*-aminophenol.

These colored compounds have not been precisely verified, but it is assumed that they are comprised of quinones, quinonimies and meri-quinonimines that are produced by the oxidation of *p*-aminophenol derivatives (Figure B4).¹³ These oxidations undoubtedly occur as side reactions during the preparation of work-up of *p*-aminophenol, which is usually formulated with low concentration of antioxidants to avoid undesired oxidation. Its oxidative property also requires a careful treatment of the industrial waste water because it could be reacted with the dissolved oxygen, which is intimately related to the aquatic life.

Finally, the number of the steps in the conventional process with chlorobenzene is 4 by excluding the additional regioisomer separation step and the subsidiary waste acids (or water) treatment process. Although chlorobenzene can be converted to the desired product via the four reaction steps- nitration, hydroxylation, reduction and acetylation, each step requires a workup and purification processes and careful treatment process for waste acids. Due to the repetitive cumbersome workup and purification processes, in-situ acetylation has been applied during the reduction of nitro group.¹⁴ The acetylation of nitrophenol in-situ does not only eliminate the interval

processes but also reduce the possibility on the formation of the previously mentioned colored impurities. Also, simple and efficient purification methods have been reported.¹⁵ However, the overall process development has not been tried for acetaminophen yet whereas new concepts have been applied to develop a process for ibuprofen, another safe analgesic.¹⁶ For example, a continuous-flow processing has been developed for the efficient and practical production of ibuprofen (Fig. B6). In the new process, the production rate was measured as 8.09 g/ hr.

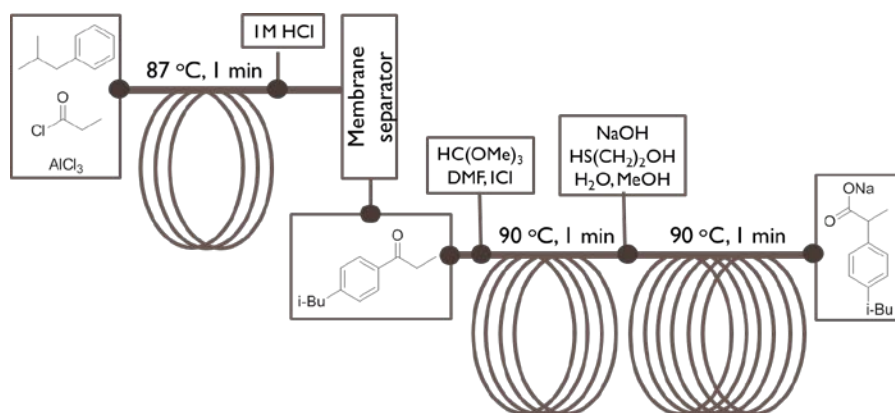


Figure B6. Continuous flow process for another analgesic, ibuprofen.

Therefore, a new efficient and eco-friendly process development has been planned to solve the chronic problems in acetaminophen production. While the previously developed processes utilize *p*-aminophenol as a key intermediate, the new process does not contain *p*-aminophenol at all. The nitration with the mixed strong acids or the reduction of nitro group in acidic aqueous solution is no more required, and the long process steps are going to be simplified via a minimum interval workup process. Moreover, the

regioselectivity issue is not considered with the new process anymore. The new process, which is seemed like an ideal process for acetaminophen, is planned based on the following previous studies on the regioselective synthesis of *p*-dihalobenzene and the metal catalyzed cross coupling reaction for C-N bond and C-O bond formation.

3. Regioselective halogenation for *p*-dihalobenzene

Halogenation, which occurs via the electrophilic substitution reaction, is essential in organic synthesis because the halogenated organic compounds are generally applied as useful synthetic intermediates for preparation of pharmaceuticals, pesticides, agricultural chemicals, and bioactive compounds. One of the well-known applications of the halogenated compounds is various cross coupling reactions which use them as useful substrates.¹⁷

The halogenation of unsaturated hydrocarbons is performed with halogen-based oxidants, including elemental halogens (X_2 , X =halides), organic or inorganic hypohalites (XO^-), hypervalent iodine reagents, dihalo-5,5-dimethylhydantoin and *N*-halosuccinimide (Fig. B7). In other words, the halogenation occurs from oxidative halogenation, in which a halide ion is oxidized to a halogenating reagent, a positive halogen (Cl^+ , Br^+ , I^+). There is an exception with fluoride in the oxidative halogenation because it is too difficult to be oxidized.

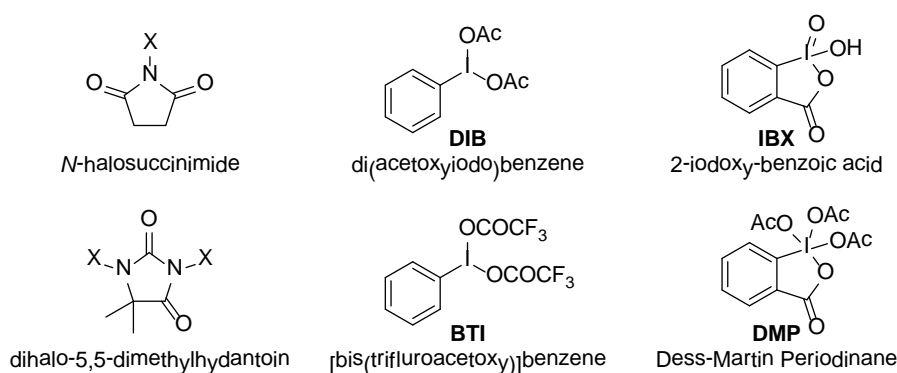
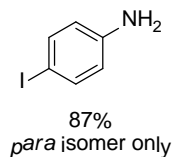
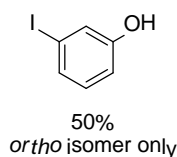
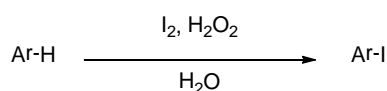


Figure B7. Common halogen-based oxidants.

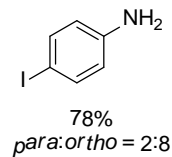
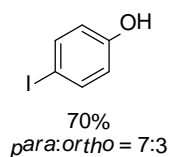
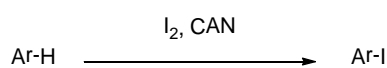
Based on the extensive applications of the halogenated compounds, synthetic

methods for halogenate unsaturated hydrocarbons or aromatic compounds have been developed. Especially, these days, green oxidative halogenation has been explored based on green chemistry perspectives, where halide salts have been applied with H_2O_2 to form the corresponding positive halogens. There have been reported some regioselective halogenation to form *p*-dihalobenzenes. The following examples are related to the regioselective halogenations for *p*-regioisomers of phenol, aniline or monohalobenzenes, which are concerned as potential starting materials in the development of a new process for acetaminophen.

(a) Iodine with oxidant

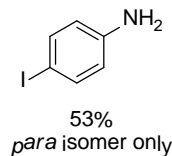
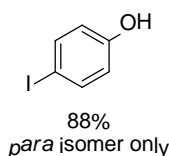
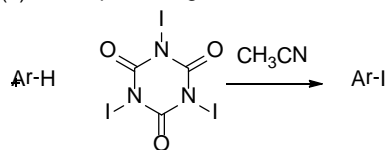


Chem. Commun., **2004**, 2614



Tetrahedron Lett., **2007**, 48, 81

(b) Electrophilic reagent



J. Braz. Chem. Soc., **2008**, 19, 1239

Figure B8. Reported halogenation reaction conditions (continued).

(c) Iodide salt with oxidant

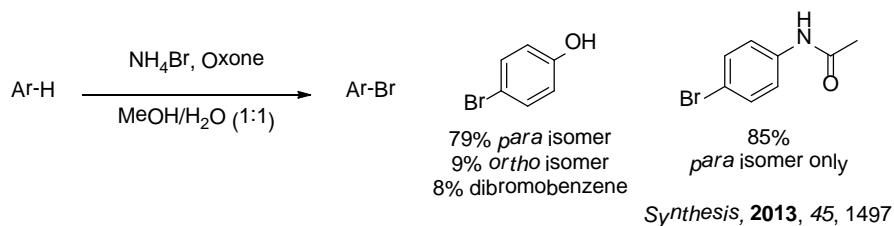
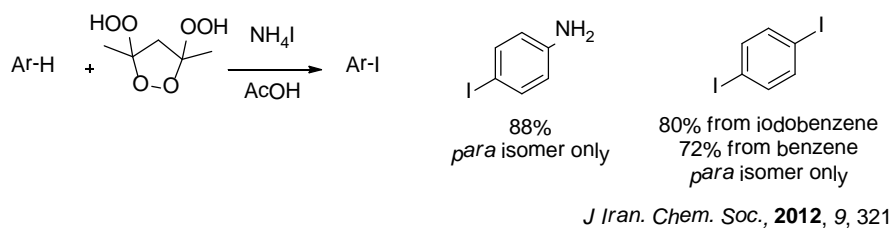
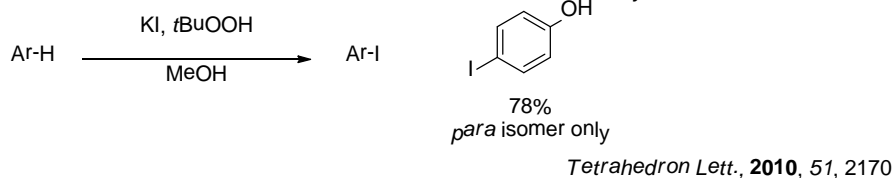
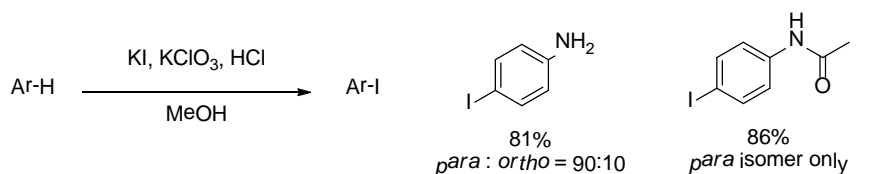
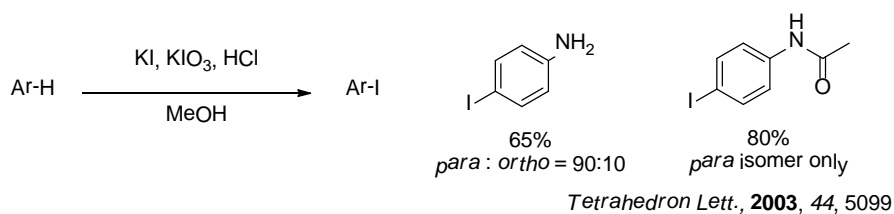
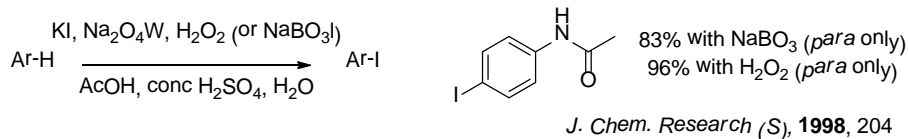
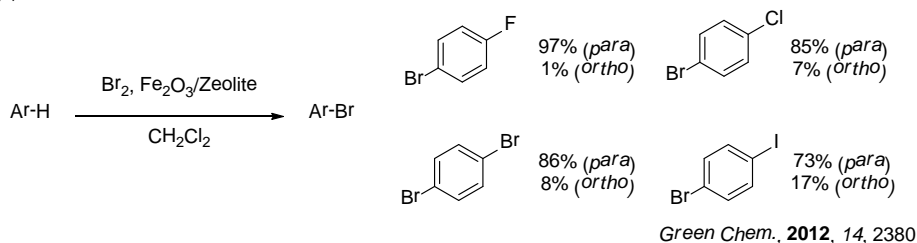


Figure B8. Previously developed halogenation reaction conditions.

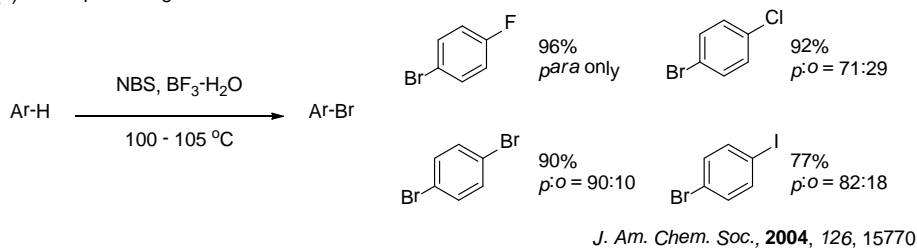
The regioselectivity on iodination of phenol or aniline is varied from *ortho*- to *para*- depending on the reaction conditions and other substituents on phenol or aniline. As like depicted in the figure B8, the ratio of *p*-isomer to *o*-isomer is increased when phenol is treated with CAN instead of H₂O₂ whereas the ratio of *p*-iodoaniline is decreased by applying CAN (Figure B8 (a)). Triiodoisocyanuric acid is successfully applied on the regioselective iodination of phenol and aniline, which are converted to *p*-isomers (Figure B8 (b)). However, halogenation with halogen or the activated reagent, such as NBS or triiodoisocyanuric acid, is considered as hazardous or non-environmental friendly.

Therefore, there have been various studies on halogenation with halide salt from green chemistry perspective. Some selective examples of halogenation with halide salt are presented in figure B8 (c). *N*-Acetylaniline is also successfully converted to *N*-acetyl-*p*-haloaniline and the yield of the desired product is usually reported in more than 80%. Moreover, both inorganic and organic halide salts are successfully applied with various oxidants. Among the selected examples, the regioselective iodination with 3,5-dihydroperoxy-3,5-dimethyl-1,2-dioxolane takes more attention because benzene is converted to *p*-diiodobenzene in 72% yield because the previously mentioned process development of acetaminophen has been planned with *p*-dihalobenzene. Based on the purpose of the study, regioselective halogenation for *p*-dihalobenzene has been searched, and some examples are shown in figure B9.

(a) Bromine with catalyst



(b) Electrophilic reagent



(c) Halide salt with oxidant

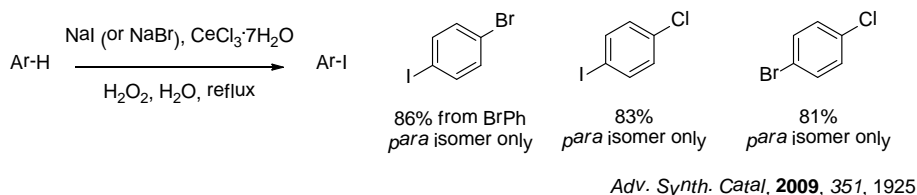
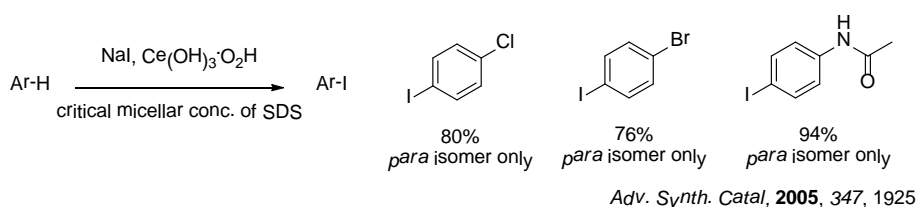
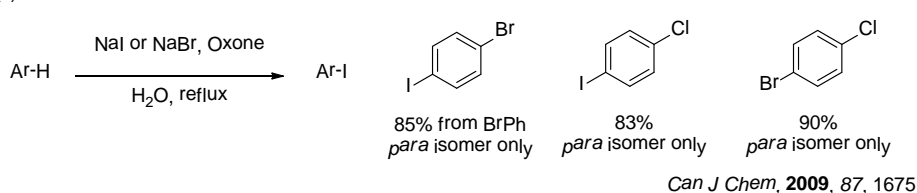


Figure B9. Reported methods for *p*-dihalobenzenes.

Similar to the previously mentioned examples with phenol and aniline, the halogen or the activated halogen reagents generally produce the mixture of *p*- and *o*- isomers, but the ratio of *p*-isomer to *o*-isomer is better than that of phenol and aniline. With halide salts, *p*-dihalobenzene has been prepared dominantly in the presence of oxidants. *N*-Acetyl-*p*-iodoaniline is synthesized in 94% yield with sodium iodide and cerium (IV) trihydroxide hydroperoxide (Fig. B9 (c)). Moreover, the practical process for the production of *p*-diiodobenzene has been developed (Fig. B10).¹⁷ In the process, benzene is regioselectively converted to *p*-diiodobenzene in the presence of Fe₂O₃/Zeolite catalysts.

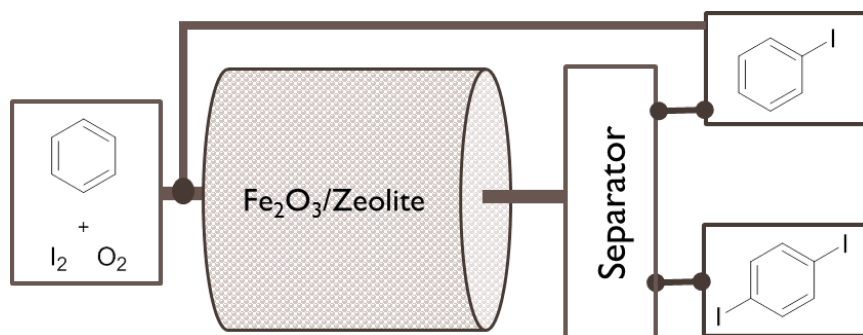


Figure B10. Practical process for *p*-diiodobenzene.

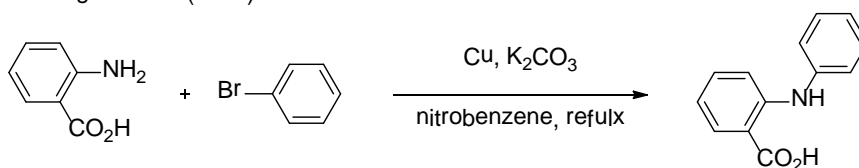
Based on the regioselective halogenations with halobenzene, phenol or aniline, a new process development of acetaminophen takes possibility of realization. The halogenated compounds, as like previously mentioned, are usually applied on various cross-coupling reactions to form new C-C, C-N or C-O bonds. The cross-coupling reaction that forms C-O or C-N bond is going

to be utilized with *p*-dihalobenzenes in the new process development of acetaminophen. Among the well-developed cross-coupling reaction, Goldberg¹⁸ reaction for C-N bond and Ullmann-type condensation¹⁹ for C-O bond are selected. Before starting the development of a new process, I took a look on the cross-coupling reactions, which was briefly summarized in the next chapter.

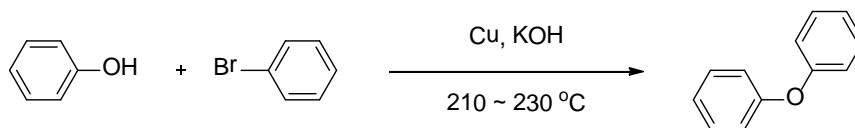
4. Goldberg and Ullmann type condensation reactions

Functionalized aromatic and heteroaromatic amines are key building blocks for the synthesis of pharmaceuticals, polymers or materials.²⁰ In recognition of their widespread importance, many synthetic methods have emerged over the years for the formation of C-O and C-N bonds, trying to overcome the shortcomings of the original Ullmann¹⁸ and Goldberg¹⁹ procedures (Scheme B4).

(a) Goldberg reaction (1906)



(b) Ullmann condensation reaction (1906)

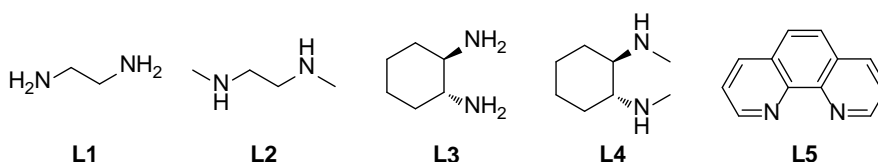


Scheme B4. Goldberg and Ullmann condensation reactions.

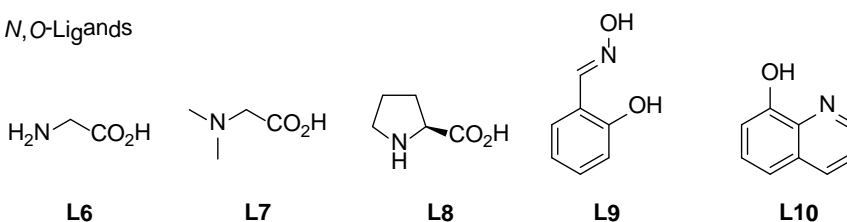
An advanced method from Goldberg reaction was reported by Buckwald who applied diamine ligands for more efficient amination with alkylamines because the use of aliphatic amines had been a long-standing problem.²² Before then, only chelating substrates, such as α - and β -amino acids and β -amino alcohols were possible to undergo the amination with aryl halides in the presence of copper catalysts. Buckwald's method was operationally simple where CuI as catalyst and ethylene glycol^{22c} as ligand in propan-2-ol were

efficiently used for the coupling of a variety of functionalized arylating agents with several amines. Besides ethylene glycol, various ligands had been developed (Fig. B11).

(a) *N,N*-Ligands



(b) *N,O*-Ligands



(c) *O,O*-Ligands

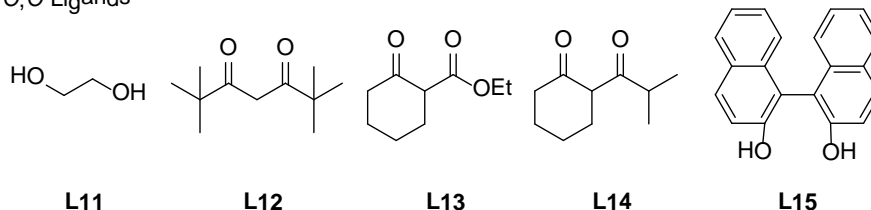
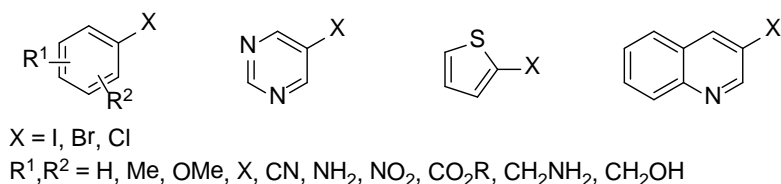


Figure B11. Various ligands for copper catalyzed amination.

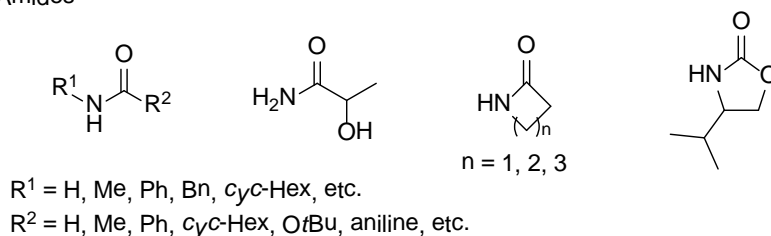
With the development of new ligands that enable reactions performed under milder conditions with improved yields, the copper catalyzed arylation of aliphatic amines had clearly become a powerful tool for the preparation of functionalized alkylarylamines. Similar to the amination, the arylation of amides had also received considerable attention over the past decade due to its high synthetic utility. The introduction of chelating ligands resulted in major improvement and dramatic softening of the reaction conditions compared to

the original procedure of Goldberg reaction.¹⁹ The following figure showed general substrates and diamine ligands, which had been successfully converted to the desired arylamides (Fig. B12).²²

(a) Aryl halides



(b) Amides



(c) Ligands

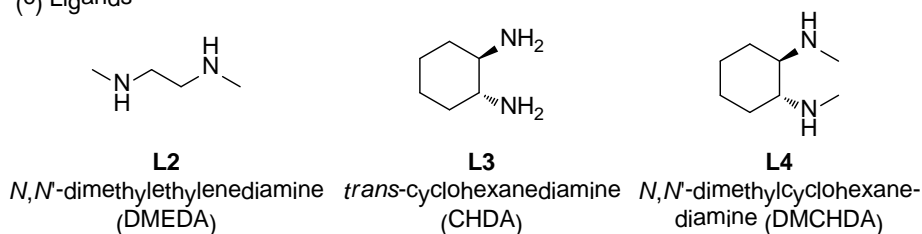


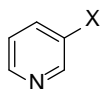
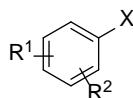
Figure B12. General substrates and ligands for arylamide synthesis.

Buckwald and co-workers extensively studied the reaction conditions and developed the inexpensive catalytic system, copper catalyzed system, based on the use of 1,2-diamine ligands (Fig. B12 (c)). Under the reaction conditions, aryl chloride and acyclic secondary amides were effectively coupled, and a variety of functional groups were tolerated in the reaction, including many that were not compatible with palladium catalysts.

Furthermore, the reaction for aryl amide had been extended to the use of other coupling partner, such as carbamates.

In the C-O bond formation, which had also been advanced as like the C-N bond formation, one of the major drawbacks of the classical Ullmann biaryl ether synthesis¹⁸ was the use of a strong base, such as *tert*-butoxide. Buckwald introduced cesium carbonate, instead of a strong base, and the preparation of diaryl ether had been performed via the improved procedure. Based on the improved procedure, ary-alkyl ether formation also had been possible with the use of milder bases, such as cesium carbonates. The first example on the reactions between a wide variety of alcohols and aryl halides had been reported by pioneering work by Hartwig and Buchwald in the presence of palladium catalysts. However, the use of palladium as catalysts limited ligand availability due to the β -hydride elimination of alcohols possessing β -hydrogens. Copper based catalysts provided more reliable activities because the intermediates derived from these catalysts did not readily undergo β -hydride eliminations. The first successful application of copper-mediated arylation of aliphatic alcohols was reported in 2002 by Buckwald, using phenanthroline or its tetramethylated derivatives (Fig. B13).²³

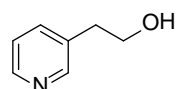
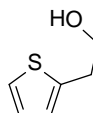
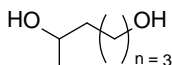
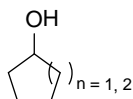
(a) Aryl halides



$X = \text{I}, \text{Br}$

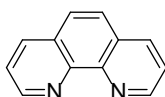
$R^1, R^2 = \text{H}, \text{Me}, \text{OMe}, \text{halides}, \text{CN}, \text{NH}_2, \text{CF}_3, \text{etc.}$

(b) Alcohols

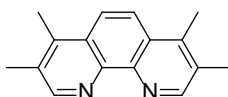


$R^1 = \text{alkyl}, \text{allyl}, \text{or propargyl}, \text{etc.}$

(c) Ligands



L5
1,10-phenanthroline



L5a
3,4,7,8-tetramethyl-
1,10-phenanthroline

Figure B13. General substrates and ligands for ether formation.

Aryl-alkyl ether could be obtained in excellent yields starting from primary, secondary, benzyl, allyl and propargyl alcohols. Like the previously mentioned, Cs_2CO_3 was applied as common base, and the reacting alcohol was usually applied as the solvent. These reaction conditions had also utilized for hydroxylation of aryl halide with K_3PO_4 in the presence of water,²⁴ contrary to that the original hydroxylation of aryl halide required hydroxide.

Based on the previous researches on the regioselective dihalobenzene preparation and two copper-catalyzed coupling reactions, a new process had been planed for more efficient and economical synthesis of acetaminophen (Fig. B14).

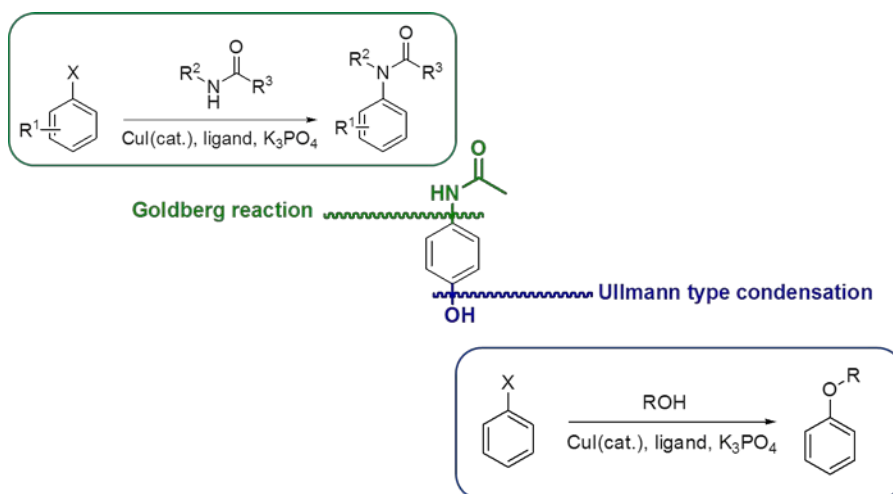


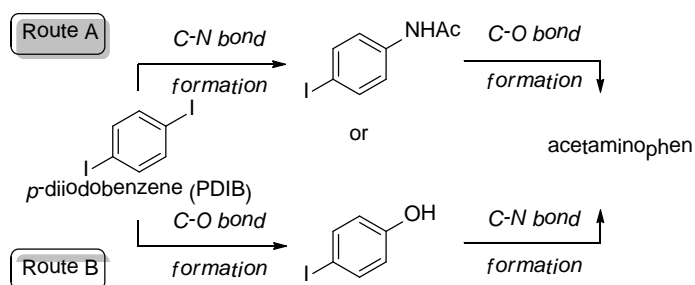
Figure B14. A new approach for efficient acetaminophen synthesis.

The previously mentioned drawbacks on the conventional process of acetaminophen would be solved with the greener and more efficient process via well-developed copper-catalyzed reactions. The detailed results of the new process development presented in the following sections.

Results and Discussions

1. The development of a new efficient process for acetaminophen

In the beginning of the synthesis of acetaminophen, two possible routes had been considered with *p*-diiodobenzene (Scheme B5). In route A, C-N bond formation was performed prior to the formation of C-O bond while the metal catalyzed hydroxylation was the first step in route B.



Scheme B5. Possible routes with *p*-diiodobenzene.

First, route A was closely considered as a potential synthetic route. There had been several examples on the diamination or diacetamidation^{25b} with dihalobenzene, and hydroxylation reaction conditions generally required hydroxide as base, which could hydrolyze *N*-acetyl moiety to amine. Based on these reported results, route A seemed like showing low possibility at first glance. However, there had much more examples on the amidation with *p*-dihalobenzenes²³ where amidated mono-halo benzenes were produced in higher yields. In addition, other milder reaction conditions for hydroxylation with aryl halide had been reported. One of them used water as the reaction solvent without any other organic solvents, and the applied base was K_3PO_4 ^{25a}

or $n\text{Bu}_4\text{NBr}$.^{25b} Under the milder reaction conditions, the desired hydroxylation with various substituted aryl halide was performed.

When the hydroxylation was performed first, however, there was a possibility of the formation of benzene,^{25d} hydroquinone,^{26a} or phenol,^{26b} instead of *p*-iodophenol²⁶ depending on the solvent, bases and oxygen source.²⁷ Although the amidation of halophenol was expected to show similar results with anisole compounds, whose yield was mostly over 80%, the route B could not be an efficient process if the undesired reactions were occurred in the hydroxylation conditions.

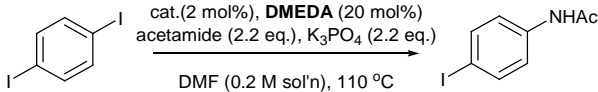
With the extensive investigation on hydroxylation and acetamidation with *p*-dihalobenzene, each first step in the route A and B had been tried with diamine ligand, *N,N'*-dimethylethylene diamine (DMEDA) in the presence of CuI and CsF as catalyst and base, respectively. Although the desired acetamidation had been performed with acetamide and *p*-diiodobenzene, the hydroxylated product, *p*-iodophenol, had not been observed after the reaction in water. Therefore, the route A, where acetamidation of *p*-dihalobenzene was followed by hydroxylation was chosen as a more potential route for acetaminophen synthesis.

2. Acetaminophen synthesis from *p*-diiodobenzene

An efficient process development for acetaminophen had been started to find an optimized conditions of the route A in Scheme B5. Among the well-developed synthetic methods for *N*-aryl amide and phenol, the combination of the inexpensive copper catalyst and diamine ligand was chosen for a new process.^{21, 22}

In order to optimize the reaction conditions with acetamide, various Cu catalysts had been applied. Besides to copper catalysts, commonly used palladium catalysts also had been applied (Table B1). Other reaction conditions were originated from the previously reported conditions.

Table B1. Catalysts screening on acetamidation

			
Entry	Catalyst	Yield	Sm recovered
1	CuI	51%	16%
2	CuBr	5%	80%
3	CuCl	10%	80%
4	CuCN	15%	40%
5	CuTC	10%	-
6	CuSO ₄	6%	12%
7	CuCl ₂	13%	63%
8	Cu(OTf) ₂	28%	41%
9	(CuOTf) ₂ Benzene	3%	80%
10	Pd(OAc) ₂ ^a	8%	90%
11	Pd ₂ dba ₃ ^a	51%	33%

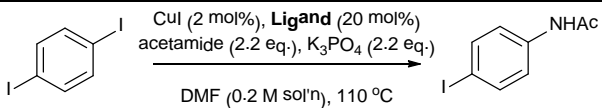
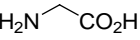
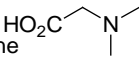
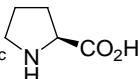
^a Cs₂CO₃ and Xantphos are used as base and ligand.

According to the results of the catalysts' screening, CuI (Entry 1) and Pd₂dba₃ (Entry 11) shown the best results with accompanying 16%, or 33% of starting material recovery, respectively. Interestingly, copper bromide (Entry 2) and copper chloride (Entry 3) were almost intact to the amidation between acetamide and *p*-diiodobenzene (PDIB). Moreover, cupric(II) triflate (Entry 8) presented better yield of the desired product, *N*-acetyl-*p*-iodoaniline, than that with cuprous(I) triflate (Entry 9), which is complexed with benzene. With CuTC (Entry 5), only 10% of the desired product was obtained without the starting material recovery.

Next, the searching for the most effective ligand had been progressed.

Glycine, proline and *N,N*-dimethyl glycine had been tested with CuI in 0.5 M DMF solution of PDIB (Table B2).

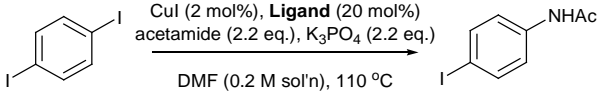
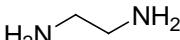
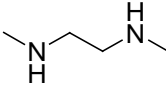
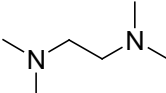
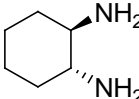
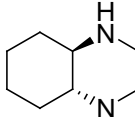
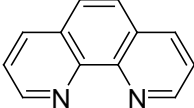
Table B2. Amino acid Ligand screening results at amidation

<div style="text-align: center;">  </div>		
Entry	Ligand	Results ^a
1	No ligand ^b	24%
2	L6 glycine 	trace
3	L7 <i>N,N</i> -dimethylglycine 	No rxn
4	L8 L-proline ^c 	product:sm = 94 : 5

^a Determined by GC; ^b Cu(OTf)₂ was used instead of CuI; ^c CsF is used as base in THF.

Although glycine and *N,N*-dimethyl glycine, which were reported as efficient ligands with copper catalysts, did not produce the desired product with acetamide, L-proline efficiently helped the copper catalyst on the acetamidation reaction. Another ligand screening study had been performed on diamine ligands (Table B3).

Table B3. Diamine Ligand screening results at amidation

<div style="text-align: center;">  </div>		
Entry	Ligand	Results ^a
1	L1^a ethylenediamine 	Product : sm = 43 : 39
2	L2^b <i>N,N'</i> -dimethylethylenediamine 	Product : sm = 94 : 6
3	 <i>N,N,N',N'</i> -tetramethylethylenediamine ^c	No rxn
4	L3^d <i>trans</i> -cyclohexanediamine 	Product : sm = 64:32
5	L4^b <i>trans</i> - <i>N,N'</i> -dimethylcyclohexanediamine 	Product : sm = 79: 6
6	L5 1,10-Phenantroline 	Product : sm = 51:16

^a Dioxane as the solvent; ^b CsF in THF was included; ^c Cu(OAc)₂ as cat.; ^d Cs₂CO₃ as base.

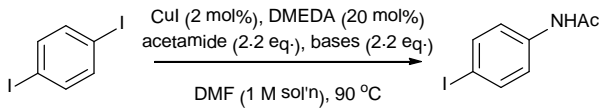
The applied diamine ligands were ethylenediamine (**L1**), *N,N'*-dimethylethylene diamine (DMEDA, **L2**), *trans*-1,2-diaminocyclohexane (**L3**), *trans-N,N'*-dimethylcyclohexane-1,2-diamine (**L4**), and 1,10-phenanthroline (**L5**), which are shown in figure B8, and *N,N,N',N'*-tetramethylethylene diamine (not shown in Fig B8). According to the results from the entry 1 to 3 in the table B3, the number of the substituted methyl on amine is seemed like a key factor on the ligand ability. Among them, the dimethyl amine showed the best conversion while the tetramethyl amine did not participate the cross coupling reaction with the copper catalyst at all. The unsubstituted diamine ligand (Entry 1) produced the desired amidated product with low conversion. The similar results had been observed with cyclohexandiamine (Entry 4) and dimethylcyclohexanediamine (Entry 5). The **L4** had converted the most of the starting materials to the desired products while almost only half of the starting material had been participate the amidation with the **L3**. With 1,10-phenanthroline, 51% of conversion had been observed.

According to the obtained results with various diamine ligands, *N,N'*-dimethyl substituted diamine ligands (Entry 2 and 5) had been selected as the most efficient ligand with copper iodide. Among the two dimethyl substituted diamine ligands, DMEDA (**L2**) was chosen for the rest of the reaction screening because it was cheaper than *trans-N,N'*-dimethylcyclohexanediamine (**L4**). Therefore, the most effective combination of catalyst and ligand in acetamidation is the combination of CuI and DMEDA (**L2**).

With the optimized results on catalyst and ligand, base screening had been

progressed in 1 M DMF solution of *p*-diiodobenzene. The results were shown in Table B4 and B5. First, the commonly applied bases, such as K_3PO_4 and Cs_2CO_3 , had been screened and the corresponding results were depicted in the table B4.

Table B4. Phosphate and carbonate bases screening at acetamidation

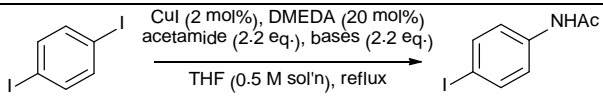
			
Entry	Base	Yield ^a	Sm recovered
1	K_3PO_4 (powder)	33%	32%
2	K_3PO_4 (granular)	45%	33%
3	K_2CO_3	23%	4%
4	Cs_2CO_3	37%	48%

^a Isolated yield.

The phosphate bases (Entry 1-2) showed better results than the carbonated bases (Entry 3-4), and the granular type of potassium phosphate (Entry 2) gave better result with 45% of the desired acetamidated product.

Although the progressed results showed the optimized results with CuI, DMEDA and granular K_3PO_4 as catalyst, ligand and base, the yield of the obtained product was still not satisfied. Therefore, the reaction solvent had changed to tetrahydrofuran (THF), having lower boiling temperature and easier to removed than DMF, and other types of base had been applied. The following table is the results of the fluoride base screening (Table B5).

Table B5. Fluoride bases screening at acetamidation

		
Entry	Base	Results ^a
1	KF	product : sm = 5 : 95
2	NaF	product : sm = 3 : 93
3	CsF	product : sm = 97 : 2
4	<i>n</i> Bu ₄ NF	product : sm = 63 : 13

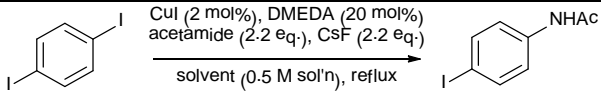
^a Determined by GC.

Potassium fluoride (Entry 1, Table B5) and sodium fluoride (Entry 2) showed the disappointed results, the majority of the starting material had been remained after the acetamidation in 0.5 M THF solution. Surprisingly, cesium fluoride (Entry 3) gave the highest conversion of *p*-diiodobenzene although tetrabutylammonium fluoride (TBAF) afforded the moderated conversion (Entry 4).

In order to find suitable solvent with the obtained results, commonly used several organic solvents had been applied to the optimized reaction conditions, shown in table B6. Ethylacetate (EA), THF and acetonitrile (MeCN) had showed high conversion ratio (Entry 2-4) under the reflux conditions, but low conversion (28%, Entry 1) was observed in DMF at 100 °C. The disappointed result in DMF might be caused by insufficient reaction temperature, but the reaction temperature screening had not been performed because DMF has high boiling point and it is hard to be removed after the reaction completed. Moreover, low reaction temperature can be an advantage on the new process development. Unlike DMF, other tested organic solvents did not cause any of

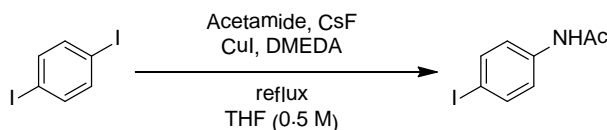
difficulties during workup process. From these reasons, THF showing highest conversion was chosen as a proper solvent at acetamidation step.

Table B6. Fluoride bases screening at acetamidation

		
Entry	Solvent	Results ^a
1	DMF ^b	product : sm = 23 : 77
2	Ethylacetate (EA)	product : sm = 78 : 22
3	THF	product : sm = 97 : 2
4	Acetonitrile (MeCN)	product : sm = 87 : 7

^a Determined by GC.; ^b Reacted at 100 °C

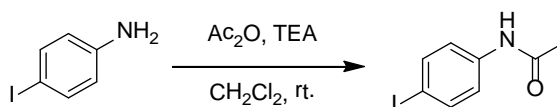
In summary, the optimized reaction conditions with *p*-diiodobenzene include the combination of CuI and DMEDA (**L2**, Figure B8) and CsF as catalyst, ligand, and base in 0.5 M THF solution of *p*-diiodobenzene (Scheme B7). After the completion of the reaction, the pure product was obtained via silica gel chromatography.



Scheme B6. Acetamidation with PDIB under the optimized conditions.

The next step for acetaminophen had been explored after the establishment of the acetamidation reaction conditions. For more efficient researches, the

starting material, *N*-acetyl-*p*-iodoaniline had been synthesized by acetylation of *p*-iodoaniline (Scheme B7).



Scheme B7. The preparation of *N*-acetyl-*p*-iodoaniline.

Like the previously mentioned in the introduction, hydroxylation with copper catalyst usually required metal hydroxide as bases. One of the previously developed reaction conditions using hydroxide as base had been applied with *N*-acetyl-*p*-iodoaniline although the hydrolysis of *N*-acetyl group had been expected. In the presence of CuI and dibenzoylmethane as catalyst and ligand, 57% of starting material was recovered, and the expected side product, 4-iodoaniline, was obtained with KOH. When $\text{CsOH} \cdot \text{H}_2\text{O}$ was added to the reaction conditions, 16% of starting material was recovered with 4-iodoaniline. However, acetanilide was observed with granule of K_3PO_4 in the presence of DMEDA as ligand instead of dibenzoylmethane.

Based on the results with K_3PO_4 , another reaction conditions had been applied in water under the microwave irradiation reaction conditions without organic solvents. In order to find suitable hydroxylation reaction conditions with *N*-acetyl-*p*-iodoaniline, potent catalysts had been applied with DMEDA (Table B7).

Table B7. Screening results of catalysts at hydroxylation of *N*-acetyl-*p*-iodoaniline

Entry	Catalyst	Results ^a
1	CuI ^b	Undesired reaction
2	CuI	product : sm = 69 : 31 ^c
3	Cu(OTf) ₂	product : sm = 66 : 19
4	(Cu(OAc) ₂)	product : sm = 66 : 17
5	FeCl ₃ (0.2 eq.) ^b	sm recovery with acetanilide
6	FeCl ₃ (0.2 eq.), Cu ₂ O (0.1 eq.) ^b	product : sm = 36 : 19

^a Determined by GC; ^b Reacted at 160 °C; ^c Isolation yield is 49%

When CuI was added to the reaction mixture, the desired product could be obtained at 180 °C (Entry 2), but at 160 °C (Entry 1) undesired reaction had been progressed. With other copper catalysts (Entry 3-4), such as Cu(OTf)₂ and Cu(OAc)₂, similar conversion had been observed comparing to the result with CuI. When FeCl₃ had been applied as a catalyst (Entry 5), starting material was recovered with the side product, acetanilide. Contrary to this, the addition of Cu₂O as an additional catalyst with FeCl₃ afforded the desired product (Entry 6). From the screening results of catalysts, acetaminophen was synthesized in 49% isolated yield with CuI as catalyst under the microwave irradiation. In order to improve the yield of the desired product, bases and organic co-solvents had been screened.

The screening results of bases had been summarized in table B8. K_3PO_4 , commonly used in the hydroxylation in water according to the previously reported conditions, was reexamined with CuI and DMEDA under microwave irradiation (Entry 1). However, the common base, K_3PO_4 , in cross coupling reaction for C-N or C-O bond formation did produce the mixture of acetanilide and aniline instead of acetaminophen. By changing the amount of CsF and concentration of the reaction mixture, the yield of acetaminophen had been increased upto 66%. Excess of CsF seemed like improving the yield of the desired product (Entry 2, 4-6, Table 8). At the low concentration, the stoichiometric amount of CsF presented acetaminophen in 66% of isolated yield.

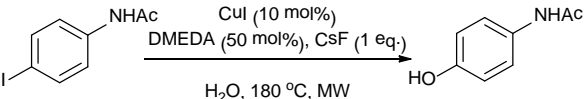
Table B8. Screening results of bases at hydroxylation of *N*-acetyl-*p*-iodoaniline

Entry	Base	Acetaminophen ^a	Acetaniline
1	K_3PO_4 (2 eq.)	the mixture of acetanilide and aniline ^b	
2	CsF (1 eq.)	48%	21%
3	CsF (1 eq.)	66%	13%
4	CsF (2 eq.)	43%	19%
5	CsF (3 eq.)	41%	12%
6	CsF (5 eq.)	55%	13%

^a Isolated yield.; ^b Determined by GC.; ^c The concentration is 0.25 M

Based on the improvement at lower concentration of the reaction mixture, the addition of organic co-solvent had been under consideration. It seemed like one of the reasons for the low yields at hydroxylation that insufficient solubility of *N*-acetyl-*p*-iodoaniline in water. However, the best result was observed in water system without any of organic solvents although the addition of organic solvent improved the solubility of *N*-acetyl-*p*-iodoaniline (Table B9).

Table B9. Screening results of co-solvent at hydroxylation of *N*-acetyl-*p*-iodoaniline

		
Entry	Co-solvent system	Results ^a
1	DMSO : H ₂ O = 1 : 1	product : acetanilide = 14 : 86
2	DMSO : H ₂ O = 1 : 3	product : acetanilide = 50 : 50
3	THF : H ₂ O = 1 : 3	product : starting mat. = 43 : 47 ^b
4	H ₂ O only	product : acetanilide = 69 : 31

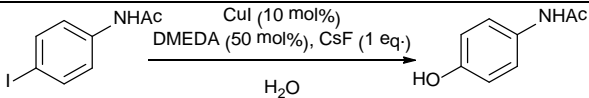
^a Determined by GC; ^b Isolation yield and reacted at 160 °C.

Although the co-solvent system of DMSO and water did not improve the previous results (Entry 1-2, Table B9), the other co-solvent system with THF and water did not produce the side product but recover the starting material in 47% isolated yield, which remained the possibility of the improved results on hydroxylation.

In summary, the hydroxylation of *N*-acetyl-*p*-iodoaniline in water had been operated at 180 °C by heating with microwave irradiation in the presence of CuI, DMEAD and CsF as catalyst, ligand and base. In industrial process,

however, the control of the reaction temperature by microwave irradiation had not been generally used. Therefore, the optimized reaction was exposed to the normal heating reaction conditions (Table B10). With the DMSO as co-solvent, majority of the starting material had been recovered at 100 °C (Entry 1). The reaction vessel was changed to sealed pressure tube from opened reaction vessel, and the reaction temperature had been increased to 110 °C or 130 °C (Entry 2-3). At the elevated reaction temperature, the desired product was obtained in 47% isolated yield.

Table B10. Results on normal heating system

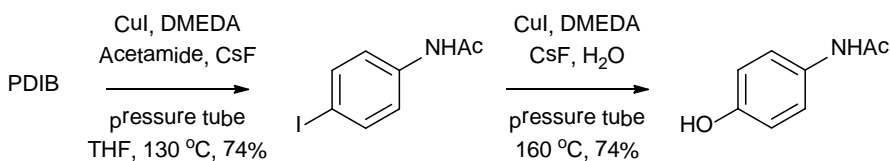
				
Entry	Concentration	Rxn vessel	Bath temp	Results ^a
1	0.5 M	Opened tube	100 °C	82% of sm
2	0.25 M	Pressure tube	110 °C	32% of sm
3	0.125 M	Pressure tube	130 °C	47%

^a Isolated yields.

In conclusion, *p*-diiodobenzene (PDIB) was successfully converted to *N*-acetyl-*p*-iodoaniline via CuI-catalyzed reaction with DMEDA as ligand with 97% of conversion and 62% of isolated yield. The following hydroxylation of *N*-acetyl-*p*-iodoaniline was resembled the first reaction conditions and the desired final product, acetaminophen, had been produced in 66% of isolation yield by the assistance of microwave. Under the normal heating system, 47% of purified acetaminophen had been synthesized.

3. Acetaminophen synthesis from *p*-dihalobenzenes

The initial optimized reaction conditions had been reexamined to increase the yield of the final product.²⁸ The following scheme shows the optimized reaction conditions with PDIB. According to the optimized results, *p*-diiodobenzene can be converted to acetaminophen in 55% overall yield via two CuI-catalyzed cross coupling reactions.



Scheme B8. The acetaminophen synthesis from *p*-diiodobenzene (PDIB).

Compared to the conventional process of acetaminophen, the newly developed two-step process had some advantages. Unlike the conventional process, any of the strong acids (H₂SO₄, and HNO₃) were not necessary. That implied the additional process step for the treatment of the waste acids was not required any more. In other words, not only the milder reaction conditions had been developed for the establishment of the eco-friendly and safe process environment, but the overall process also had become simple by changing the reaction circumstances. The other difference was the number of steps. According to the conventional process, at least 4 to 5 steps were required including the separation step to the *p*-regioisomer, but only two steps were necessary in the present process.

However, the present process showed a limitation, which had not been improved even by the extensive screening the reaction. The similar

disappointed result with PDIB²⁹ had been reported on copper catalyzed cross coupling reactions even though the fundamental reason had not been identified yet. Therefore, we considered 4-bromiodobenzene as an alternative potent starting material based on the fact that 4-bromiodobenzene had been applied to the C-N bond cross coupling reaction as a starting material and usually showed high yield (80~94%)²² in order to strengthen the competitiveness of the two-step process.

The same synthetic route, which was applied for PDIB (Scheme B9), had been reexamined with a new starting material, *p*-bromiodobenzen. Before starting the optimization of the reactions, we expected better results based on the previous results that *p*-bromiodobenzen had been more frequently applied to amidation reaction and shown better results than PDIB. Moreover, that the previously reported results on the hydroxylation of both bromo- and iodo-aryl compounds showed the similar yields was also supported our expectation for better result with the new starting material.

Although the optimal conditions had been reported with PDIB²⁶, all of the reaction conditions had been screened again. The results of the amidation with *p*-bromiodobenzene were presented in table B11. First of all, various cupric or cuprous catalysts were applied with the previously applied diamine ligands for the cross coupling reaction with acetamide. Distinct from the results with PDIB, there was chemoselectivity issue with 4-bromiodobenzene. Interestingly, the ratio of *N*-acetyl-*p*-bromoaniline to *N*-acetyl-*p*-iodoaniline was varied depending on the reaction conditions. For example, *N*-acetyl-*p*-bromoaniline was afforded as the sole product when the reaction was performed by refluxing THF solution of reaction mixture under N₂

atmosphere while the mixture of *N*-acetyl-*p*-bromoaniline and *N*-acetyl-*p*-iodoaniline was obtained in the pressure tube at the elevated reaction temperature (130 °C) although the other reaction conditions were identical. However, it was unnecessary to dispute about the chemoselectivity because it would not be an issue unless the yields on the hydroxylation of *N*-acetyl-*p*-bromo- and iodo-aniline had a big difference. Fortunately, in the previous study with PDIB,²⁸ the yield on the hydroxylation of *N*-acetyl-*p*-bromoaniline (75%) was approximately equal to that of *N*-acetyl-*p*-iodoaniline (74%).²⁸

Among the examined copper catalysts (Entry 1-10) under the condition A where 2.2 equivalent of acetamide and CsF were applied, Cu powder and CuI showed highest yields (Entry 1-2) while CuSO₄ and Cu(OTf)₂ (Entry 9-10) showed less effective catalytic reactivities in amidation with *N,N'*-dimethylethylene diamine (DMEDA) as the ligand. Interestingly, the tested cuprous catalysts (Entry 2-6), such as copper iodide, copper bromide, copper cyanide and cuprous oxide, were presented higher yields than the results with cupric catalysts (Entry 8-10) except for cupric oxide (Entry 8).

Table B11. Optimization of the reaction conditions for acetamidation

Entry	Catalyst	Ligand	Base	Yield (%) ^a	
				Condition A ^b	Condition B ^c
1	Cu ^d	DMEDA	CsF	80	93 ^e
2	CuI	DMEDA	CsF	82	93 ^e
3	CuBr	DMEDA	CsF	73	67 ^f
4	CuCl	DMEDA	CsF	72	72 ^e
5	CuCN	DMEDA	CsF	79	63
6	CuTC	DMEDA	CsF	65	69
7	Cu ₂ O	DMEDA	CsF	68	34
8	CuO	DMEDA	CsF	77	92 ^f
9	CuSO ₄	DMEDA	CsF	54	66 ^e
10	Cu(OTf) ₂	DMEDA	CsF	49	74 ^e
11	CuI	<i>N</i> -Me-EDA	CsF	62	- ^g
12	CuI	EDA	CsF	64	- ^g
13	CuI	CHDA	CsF	57	- ^g
14	CuI	DMCHDA	CsF	74	- ^g
15	CuI	1,10-phenanthroline	CsF	55	- ^g
16	CuI	DMEDA	K ₃ PO ₄	76	- ^g
17	CuI	DMEDA	KF	32	- ^g
18	CuI	DMEDA	Cs ₂ CO ₃	91 ^e	92 ^e
19	CuI	DMEDA	CsBr	7 ^h	- ^g
20	CuI	DMEDA	CsI	No rxn ⁱ	- ^g

^a Isolation yields, which was calculated based on the ratio of *N*-acetyl-*p*-iodoaniline and *N*-acetyl-*p*-bromoaniline, which was measured by ¹H NMR.; ^b 2.2 eq. of acetamide and base were applied.; ^c 4 eq. of acetamide and base were applied.; ^d Cu powder was applied as a catalyst.; ^e *N*-Acetyl-*p*-bromoaniline was obtained as a sole compound.; ^f The observed selectivity on *N*-acetyl-*p*-bromoaniline to *N*-acetyl-*p*-iodoaniline was more than 15:1 by ¹H NMR. ; ^g The reaction had not been tested.; ^h 92% of the starting material was recovered.; ⁱ 99% of the starting material was recovered.

Interestingly, the improvement of the yield was observed under the condition B in which 4.0 equivalents of acetamide and CsF were added except for CuBr, CuCN, and Cu₂O (Entry 3, 5, and 7). Under the condition B, not only the improved results of the desired amidation reaction had been observed, but the chemoselectivity in most of reactions also had been changed with affording *N*-acetyl-*p*-bromoaniline as a single product.

With the suitable copper catalyst, CuI, well-known diamine ligands (Fig. B12) were applied to optimize the reaction conditions. The diamine ligands with primary amines (Entry 11-13) less effectively helped the copper catalyst compared to the secondary diamine ligands (Entry 2 and 14). *trans*-Cyclohexane-1,2-diamine (CHDA) and 1,10-phenanthroline were appeared as insufficient ligands under the acetamidation with the selected copper iodide catalyst (Entry 13, 15). The best combination of CuI and DMEDA was applied for the rest of the optimization of other reaction conditions, such as base and solvent. Surprisingly, Cs₂CO₃ presented the highest yield and *N*-acetyl-*p*-bromoaniline was afforded as the sole product (Entry 18). While K₃PO₄, which was a commonly used base on C-N bond formation with copper catalyst and diamine ligand, showed moderate yield (Entry 16), KF only furnished 32% of the desired product (Entry 17). Based on the good results with CsF and Cs₂CO₃, CsBr and CsI were added as the base respectively, but the most of the starting material were recovered in both cases (Entry 19-20). In order to find a more suitable organic solvent with the other optimized reaction conditions, organic solvents having low boiling points had been applied, such as ethyl acetate, acetonitrile, and 1,4-dioxane, and it had been revealed that tetrahydrofuran (THF) was the best choice among them. The

reason why an organic solvent having low boiling point had been searched was for a more advanced process development for acetaminophen, which would be discussed at the following section. In summary, the acetamidation of *p*-bromiodobenzene were optimized with the excess amount of acetamide and CsF and the combination of CuI, and DMEDA, or Cu powder with DMEDA in THF at 130 °C for 24 h.

To study the scope of the procedure, the reaction of *p*-substituted halobenzenes with acetamide was examined at the optimal conditions (Table B12). Most of examined *p*-substituted halobenzenes were coupled with acetamide to give the desired products in good yields. Iodobenzenes bearing various substituted were successfully converted to *N*-acetylamido-*p*-substituted benzenes (Entry 1-7). However, in spite of various efforts to improve the yield with *p*-diiodobenzene, it was not fully converted to the desired product where starting material was recovered in 9% at the end of the reaction (Entry 1). Interestingly, the amidation of iodophenol (Entry 7), which had been considered as an alternative process for the route B in scheme 2, quantitatively afforded the desired final product acetaminophen in a step. *p*-Substituted bromobenzenes were also successfully converted to the desired products, but the lower yield than that of iodobenzenes were observed (Entry 8-13). 4-Bromonitrobenzene showed the highest yield among the examined bromobenzenes (Entry 11) while 4-bromophenol furnished acetaminophen in low yield (Entry 13). Low efficiency of acetamidation with *p*-dichlorobenzene was observed (Entry 14).

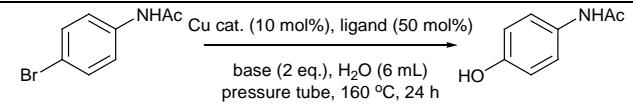
Table B12. Acetamidation with various halobenzenes

$ \begin{array}{ccc} \text{R}^1\text{-C}_6\text{H}_4\text{-R}^2 & \xrightarrow[\text{THF, 130 } ^\circ\text{C}]{\text{CuI (2 mol\%), DMEDA (20 mol\%)}, \text{acetamide, CsF, pressure tube}} & \text{R}^1\text{-C}_6\text{H}_4\text{-NHAc} \end{array} $			
Entry	R ¹	R ²	Yield %
1	I	I	69
2	Br	I	93
3	Cl	I	99
4	F	I	99
5	NO ₂	I	99
6	MeO	I	99
7	HO	I	99
8	Br	Br	57
9	Cl	Br	53
10	F	Br	43
11	NO ₂	Br	88
12	MeO	Br	53
13	HO	Br	16
14	Cl	Cl	17

establishment of the next hydroxylation step in order to complete the new eco-friendly process for acetaminophen. Similar to the previous study with PDIB, copper catalyst and diamine ligand were employed and the copper catalyzed phenol synthesis in water kept explored instead of the mixed solvent system with organic solvent, such as dimethylsulfoxide(DMSO). Based on the previously established hydroxylation conditions with *N*-acetyl-*p*-iodobenzene

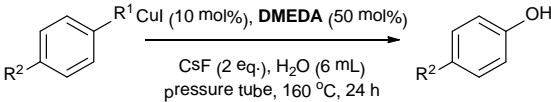
(2), we tried to find more compatible reaction conditions for the hydroxylation of *N*-acetyl-*p*-bromoaniline (**3**), which had been tested under the previously optimized reaction conditions in the previous study.¹⁹ The previous microwave reaction conditions were reexamined with *N*-acetyl-*p*-bromoaniline (**3**), and the final product acetaminophen was obtained in 73% yield at the lowered reaction temperature (120 °C) under the same previous reaction conditions except for the temperature. Because the better yield was observed under the normal heating reaction conditions with pressure tube according to our previous study, the reaction conditions were reexamined under the normal heating system. The results of the optimization were stated in table 3. The selected copper catalysts and diamine ligands, which showed better results at the amidation step, were tested. Copper iodide showed the highest isolated yield of acetaminophen (Entry 1), and other selected copper catalysts also presented good yields (Entry 2-5). In accordance with the ligand screening results at the amidation step, DMEDA furnished the best results (Entry 1). Among other diamine ligands tested, *trans*-*N,N'*-dimethylcyclohexane diamine (DMCHDA) also showed a pleasing result on hydroxylation with water (Entry 8). However, in contrast with the results of base screening in the previous amidation step, Cs₂CO₃ and K₃PO₄ were scarcely afforded the final product (Entry 11, 12) while KF furnishes acetaminophen in 77 % yield (Entry 13). CsBr and CsI (Entry 9, 10) did not provide any good results in this step again. In summary, the optimal result on the hydroxylation was given in water at 160 °C by the combination of CuI, DMEDA and CsF as the catalyst, ligand and base respectively.

Table B13. Optimization of the reaction conditions for hydroxylation

				
Entry	Catalyst	Ligand	Base	Yield %
1	CuI	DMEDA	CsF	85
2	Cu powder	DMEDA	CsF	80
3	CuCN	DMEDA	CsF	65
4	Cu ₂ O	DMEDA	CsF	70
5	CuO	DMEDA	CsF	70
6	CuI	<i>N</i> -Me-EDA	CsF	70
7	CuI	EDA	CsF	64
8	CuI	DMCHDA	CsF	76
9	CuI	DMEDA	CsBr	38
10	CuI	DMEDA	CsI	20
11	CuI	DMEDA	Cs ₂ CO ₃	Trace
12	CuI	DMEDA	K ₃ PO ₄	Trace
13	CuI	DMEDA	KF	77

To study the scope of the established hydroxylation conditions, other potential intermediates for acetaminophen, including *N*-acetyl-*p*-haloaniline and *p*-substituted halobenzenes, were examined at the optimal reaction condition with water. The results of the study were summarized in table B14.

Table B14. CuI-catalyzed hydroxylation with various halobenzenes

			
Entry	R ¹	R ²	Yield %
1	I	NHAc	78
2	Br	NHAc	85
3	Cl	NHAc	73
4	F	NHAc	-
5	I	NH ₂	45
6	Br	NH ₂	74
7	Cl	NH ₂	60
8	F	NH ₂	-
9	I	NO ₂	28
10	Br	NO ₂	29
11	Cl	NO ₂	14
12	F	NO ₂	-

Most potent intermediates for acetaminophen, *N*-acetyl-*p*-haloanilines (Entry 1-3), were successfully furnished the desired product in good yield. Interestingly, *N*-acetyl-*p*-iodoaniline showed lower yield than that of *N*-acetyl-*p*-bromoaniline due to the undesired side reaction, de-halogenation. With *N*-acetyl-*p*-iodoaniline, *N*-acetylaniline was obtained in 5% yield.

Including the *N*-acetyl-*p*-fluoroaniline (Entry 4), all of the examined *p*-substituted fluoroaniline remained intact under the copper catalyzed hydroxylation (Entry 3, 7, and 11). While *p*-aminophenol, well-researched intermediate for acetaminophen, was synthesized from the *p*-haloaniline in

moderate to good yields (Entry 5-7), *p*-nitrophenol that was one of precursors for *p*-aminophenol was obtained in low yields from *p*-nitrohalobenzene.

In summary, acetaminophen had been efficiently synthesized in 79% yield from *p*-bromiodobenzene via the suggested two-step process. The two-step process was consisted with two copper catalyzed cross coupling reactions between the dihalobenzene and acetamide or water, and the each of the reaction conditions was resemble each other. From that point, we planned to simplify the established two-step process. We expected that the two-step process could be a one-pot process by simplifying the interval workup and purification procedures.

4. A New one-pot process for acetaminophen

Based on the extensive screening results on acetamidation and hydroxylation with *p*-dihalobenzenes, the optimized two-step process was changed to be suitable for the new purpose. The first thing to accomplish the one-pot process was to make each of the reaction conditions in the two-step process similar. Among the optimal bases (CsF and Cs₂CO₃) at the amidation step, CsF was selected because Cs₂CO₃ did not produce acetaminophen under the copper catalyzed hydroxylation conditions with water. Therefore, the determined reagents for the one-pot process were CsF and the combination of CuI and DMEDA as base, catalyst and its ligand.

First, we hypothesized that the hydroxylation would be progressed by the addition of water to the condensed reaction mixture, which was readily prepared by the removal of the solvent right after the amidation reaction was completed. However, the desired *a* was obtained only in 44% yield with the mixture of *N*-acetyl-*p*-bromiodobenzene and acetanilide. The prolonged reaction time (48 h) at the hydroxylation step did also provide the same result (46% isolation yield). From these disappointed results, we assumed that the loss of diamine ligand could be accompanied with the removal of solvent, so the additional DMEDA (0.2 equiv.) was applied when the water was added to the condensed reaction mixture. As the result of it, acetaminophen was synthesized in the improved yield (57%), but the new one-pot process did not seem to be efficient as much as the established two-step process due to the low yield. Therefore, we tried the hydroxylation without any treatments except for the addition of water to the reaction mixture right after the amidation was completed. Surprisingly, the yield of the synthesized *N*-acetyl-

p-aminophenol had been increased to 74%, which was the similar result with that from the two-step process (79%) under the condition B in table B11.

Table B15. One-pot procedure for acetaminophen

<div style="text-align: center;"> </div>				
Entry	R ¹	R ²	Yield (%)	
			One-pot	Two-pot
1	Br	I	74	79
2	Br	Br	50	48
3	Br	Cl	37 ^a	42
4	I	Cl	38 ^b	72
5	I	I	35 ^c	54

^a. 32% of *N*-acetyl-*p*-chloroaniline was obtained.;

^b. 24% of *N*-acetyl-*p*-chloroaniline was obtained.;

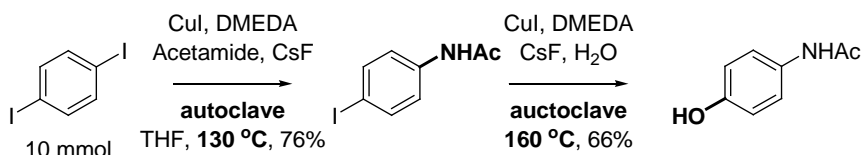
^c. 13% of *N*-acetyl-*p*-iodoniline was obtained.

The simplified one-pot procedure was examined with *p*-dihalobenzenes for the scope of the new procedure. The results of the sequential amidation and hydroxylation without the interval purification step were stated in table B15 with the results from the two-step process. *p*-Dibromobenzene and *p*-bromochlorobenzene were converted to the desired *N*-acetyl-*p*-aminophenol in 50% and 37% yield (Entry 2-3), which were the almost same results from two-step process. However, the results from one-pot procedure with *p*-chloriodobenzene (Entry 4) or *p*-diiodobenzene (PDIB, Entry 5) did not reach to the results from two-step process as affording the *N*-acetyl-*p*-haloaniline in 24% and 13% respectively.

5. Acetaminophen production via the Cu-catalyzed process

p-Diiodobenzene was successfully converted to the desired acetaminophen via the suggested two Cu-catalyzed reactions (Scheme B8). Under the optimized reaction conditions in normal heating system, each Cu-catalyzed reaction afforded the desired products in 74% yields, respectively.

In order to check whether the new process could be applied as an industrial process, the acetamidation was performed with 10 mmol of *p*-diiodobenzene in autoclave instead of the pressure tube (Scheme B9). The desired *N*-acetyl-*p*-iodobenzene was synthesized in 53% yield with 31% of starting material recovery. The hydroxylation also conducted at the same scale (10 mmol) to produce the desired acetaminophen, and the desired analgesic was produced in 46% yield with the 30% of the starting material recovery.



Scheme B9. Acetaminophen production via Cu-catalyzed process.

Although the starting materials were remained in large-scale, the overall yield based on the starting material recovery was 50%, which was similar to the result in small scale. Because the remained starting material could be recycled or recovered, the developed Cu-catalyzed process was considered as a suitable industrial process for acetaminophen.

The suggested Cu-catalyzed process had an economical feasibility issue

because the new process required a relatively expensive starting material, *p*-diiodobenzene. In the conventional process for acetaminophen, phenol or chlorobenzene was used as the starting material. However, the issue could be solved by the recovery of iodine from the resulting mixture after the reactions.³⁰ According to the previous study on the phenylenediamine production from *p*-diiodobenzene, iodide salts from the reaction mixture were recovered with H_2O_2 and recycled to produce *p*-diiodobenzene, and the yield of the recovered iodine was 98%.

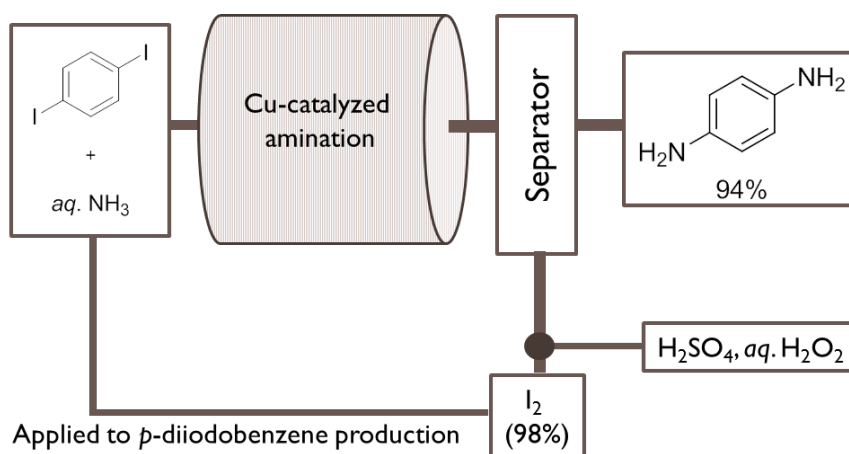


Figure B15. Recovery of I_2 in the phenylenediamine production.

Conclusion

It had been developed that a practical and efficient process for acetaminophen from *p*-dihalobenzenes. The developed process does not only allow eco-friendly reaction environments via the inexpensive copper catalyzed reactions but also solve the conventional issues on acetaminophen production including the formation of regio-isomers and the inevitable process steps, such as the waste acids treatment step, and a separation step for the desired *p*-regioisomers. From the established two-step process, *p*-bromiodobenzene was successfully converted to *N*-acetyl-*p*-aminophenol in 79% yield. Furthermore, the two-step process was simplified to the one-pot process for more practical and economical process. The simplified one-pot process also successfully converted *p*-bromiodobenzene to the desired acetaminophen in 74% yield.

Experimental Details

General Procedure. All reactions were carried out in oven-dried pressure tube with magnetic stirring. All materials were obtained from commercial sources and were used without further purification. Reaction solvents, tetrahydrofuran and distilled water, were sparged with argon prior to use. The reactions were monitored with a SiO₂ TLC plate under UV light (254 nm). Column chromatography was performed on silica gel 60 (70-230 mesh). Melting points were measured on a Meltemp apparatus in open capillary tubes. ¹H and ¹³C NMR spectra were measured at 400 MHz and 100 MHz, respectively in DMSO-d₆ with Bruker Avance-400 unless stated otherwise. The ¹H NMR spectroscopic data were reported as follows in ppm (δ) from the 2.5 resonance of DMSO-d₆: chemical shift (multiplicity, integration, coupling constant in Hz). The ¹³C NMR spectra data were referenced with the 39.51 resonance of DMSO-d₆. Low and high resolution mass spectra were measured by the FAB ionization method and analyzed by magnetic sector mass analyzer.

General procedure for amidation of *p*-substituted halobenzenes with acetamide under Condition A

To a pressure tube which was backfilled with N₂ *p*-dihalobenzenes or *p*-substituted halobenzene, (1 mmol), acetamide (236 mg, 4.0 mmol), CsF (608 mg, 4.0 mmol) and CuI (3.8 mg, 0.02 mmol) were added, and then the immediately sparged THF (4 mL) was added to the pressure tube. The tube

was sealed carefully right after the addition of *N,N'*-dimethylethylenediamine (DMEDA, 22 μ L, 0.2 mmol). The sealed pressure tube was heated to 130 °C and vigorously stirred for 24 h. After cooling back to room temperature, the mixture was diluted with EtOAc (20 mL), and an aqueous saturated solution of NaCl (20 mL) was added to the mixture. The resulting mixture was separated and the aqueous layer was extracted with EtOAc (20 mL \times 3). The combined organic layers were dried over MgSO₄, filtered and concentrated under the reduced pressure. The obtained organic residue was purified by silica gel chromatography (hexane/EtOAc=2:1) to afford the desired *N*-acetyl-*p*-haloaniline or *N*-acetyl-*p*-substituted aniline.

***N*-(4-Iodophenyl)acetamide**

; white solid; mp 185~186 °C (lit.²² 182~184 °C); 69% (from *p*-diiodobenzene); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.03 (s, 3H), 7.41 (d, 2H, *J*=8.8), 7.61 (d, 2H, *J*=8.8), 10.02 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 24.0, 86.2, 121.1, 137.2, 139.1, 168.4. HRMS (FAB) calcd for C₈H₈INO 261.9729 ([M+H]⁺), found 261.9723.

***N*-(4-Bromophenyl)acetamide**

; white solid; mp 168~170 °C (lit.²³ 166~167 °C); 93% (from *p*-bromoiodobenzene); ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.04 (s, 3H), 7.46 (d, 2H, *J*=9.0), 7.55 (d, 2H, *J*=9.2), 10.05 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 24.0, 114.4, 120.8, 131.4, 138.6, 168.4. HRMS (FAB) calcd for C₈H₈BrNO 213.9868 ([M+H]⁺), found 213.9866.

***N*-(4-Fluorophenyl)acetamide**

; brown solid; mp 153~154 °C (lit.²⁴ 152 °C); 99% (from *p*-fluoriodobenzene); ¹H NMR (400 MHz, DMSO-d₆) δ 2.03 (s, 3H), 7.10 (t, 2H, *J*=9.2), 7.59 (dd, 2H, *J*=5.2, 9.2), 10.00 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 23.8, 115.1 (d, *J*=22.0), 120.7 (d, *J*=7.0), 135.7 (d, *J*=2.0), 157.8 (d, *J*=237); HRMS (FAB) calcd for C₈H₈FNO 154.0668 ([M+H]⁺), found 154.0665.

***N*-(4-Nitrophenyl)acetamide**

; yellow solid; mp 215~216 °C (lit.²³ 215~216 °C); 99% (from *p*-nitroiodobenzene); ¹H NMR (400 MHz, DMSO-d₆) δ 2.12 (s, 3H), 7.82 (d, 2H, *J*=9.2), 8.21 (d, 2H, *J*=9.2), 10.57 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 24.1, 118.5, 124.8, 142.0, 145.4, 169.3; HRMS (FAB) calcd for C₈H₈N₂O₃ 181.0613 ([M+H]⁺), found 181.0618.

***N*-(4-Methoxyphenyl)acetamide**

; white solid; mp 127~128 °C (lit.²³ 128~130 °C); 99% (from *p*-iodoanisole); ¹H NMR (400 MHz, DMSO-d₆) δ 1.99 (s, 3H), 3.71 (s, 3H), 6.85 (d, 2H, *J*=9.2), 7.46 (d, 2H, *J*=9.2), 9.76 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 23.7, 55.1, 113.8, 120.6, 132.6, 155.1, 167.8; HRMS (FAB) calcd for C₉H₁₁NO₂ 166.0868 ([M+H]⁺), found 166.0873.

General procedure hydroxylation of *N*-acetyl-*p*-halobenzene

To a pressure tube backfilled with N₂, *N*-acetyl-*p*-halobenzene (1 mmol), CsF (304 mg, 2.0 mmol) and CuI (19.1 mg, 0.1 mmol) was added, and then the

immediately sparged H₂O (6 mL) was added to the pressure tube. The tube was sealed carefully right after the addition of DMEDA (54 □L, 0.5 mmol). The sealed pressure tube was heated to 160 °C and vigorously stirred for 24 h. After the cooling back to the room temperature, the mixture was diluted with EtOAc (20 mL) and then the aqueous layer was saturated with NH₄Cl salt. The resulting mixture was separated and the aqueous layer was extracted with EtOAc (20 mL×4). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The organic residue was purified by silica gel chromatography (hexane/EtOAc = 1:4) to afford the desired acetaminophen.

***N*-(4-Hydroxyphenyl)acetamide**

; white solid; mp 168~170 °C (lit.²³ 166~167 °C); 78% (from *N*-(4-iodophenyl)acetamide); ¹H NMR (400 MHz, DMSO-d₆) δ 1.99 (s, 3H), 6.69 (d, 2H, *J*=9.0), 7.35 (d, 2H, *J*=9.0), 9.12 (s, 1H), 9.63 (s, 1H); ¹³C NMR (100 MHz, DMSO-d₆) δ 23.7, 115.0, 120.9, 131.0, 153.2, 167.6; HRMS (FAB) calcd for C₈H₉NO₂ 152.0712 ([M+H]⁺), found 152.0714.

General procedure for one-pot procedure for acetaminophen from *p*-dihalobenzenes

To a pressure tube which was backfilled with N₂, *p*-dihalobenzenes (1 mmol), acetamide (62 mg, 1.05 mmol), CsF (304 mg, 2.0 mmol) and CuI (3.8 mg, 0.02 mmol) were added, and then the immediately sparged THF (4 mL) was added to the pressure tube. The tube was sealed carefully right after the addition of DMEDA (22 μL, 0.2 mmol). The sealed pressure tube was heated to 130 °C and vigorously stirred for 24 h. After cooling back to room

temperature, the sparged H₂O (6 mL) was added to the reaction mixture. The tube was sealed carefully, and the sealed pressure tube reacted at 160 °C with vigorous stirring for 24 h. After the cooling back to the room temperature, the mixture was diluted with EtOAc (20 mL) and then the aqueous layer was saturated with NH₄Cl salt. The resulting mixture was separated and the aqueous layer was extracted with EtOAc (20 mL×4). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The organic residue was purified by silica gel chromatography (hexane/EtOAc = 1:4) to afford the desired acetaminophen.

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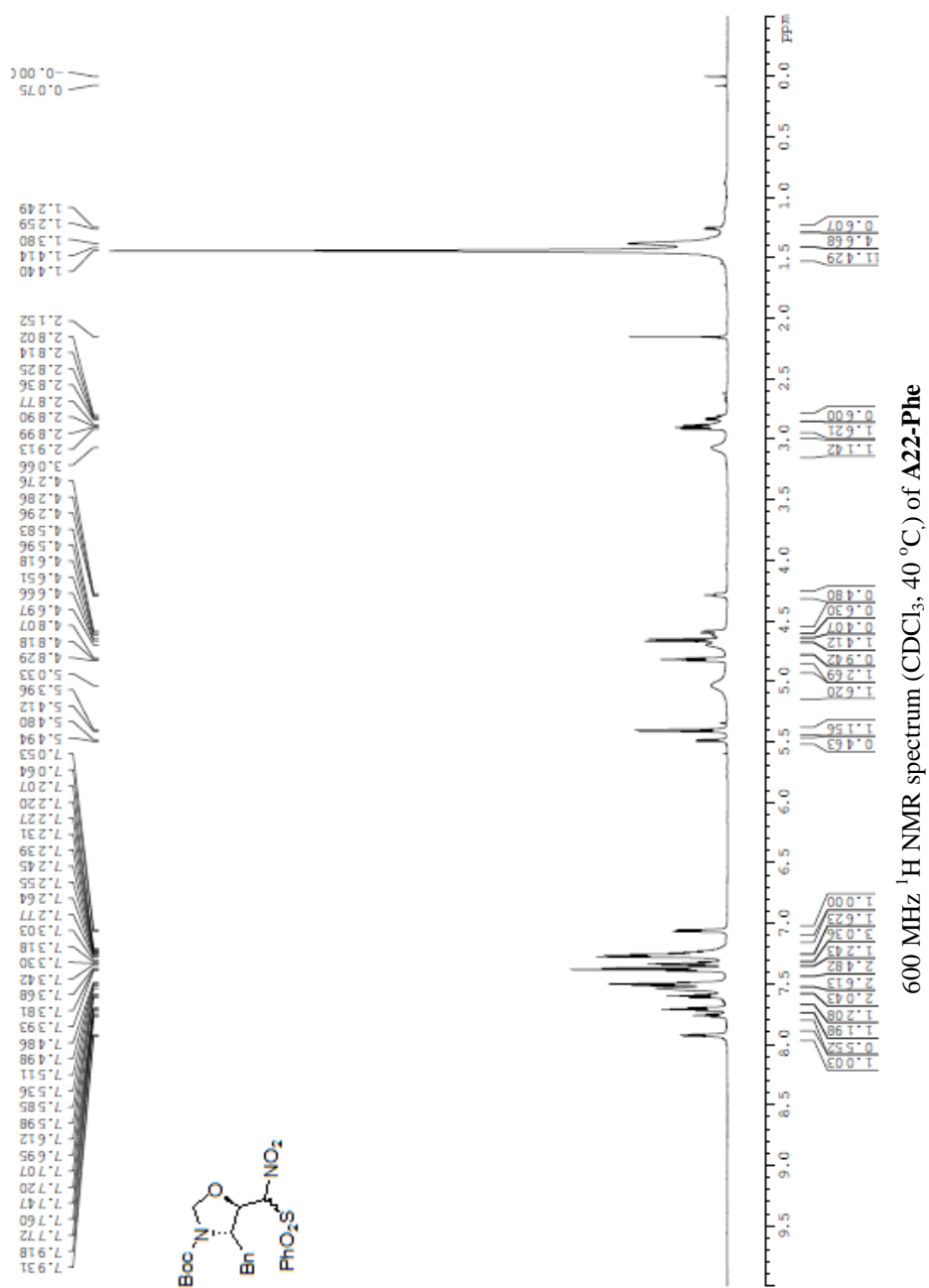
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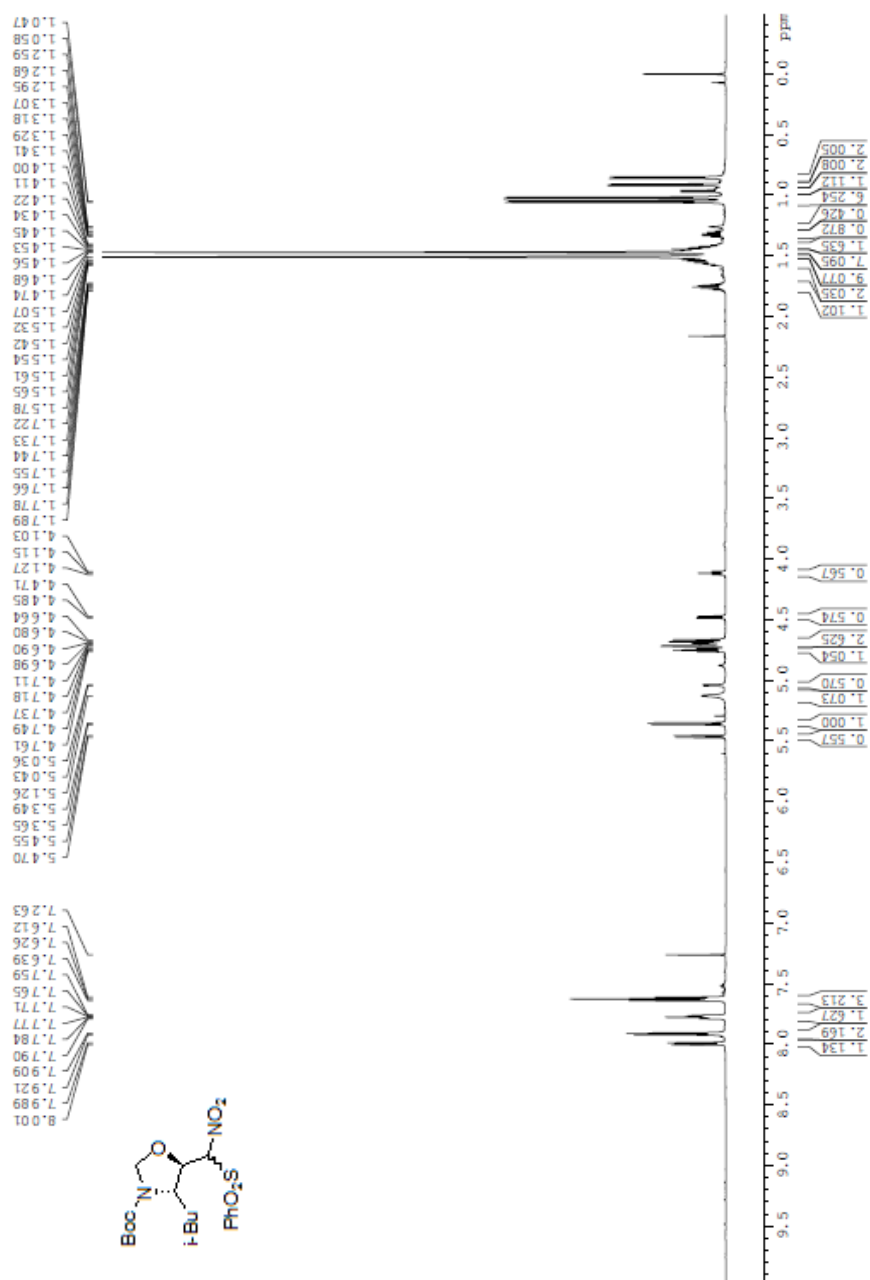
Appendices

List of ¹H-NMR Spectra of Selected Compounds

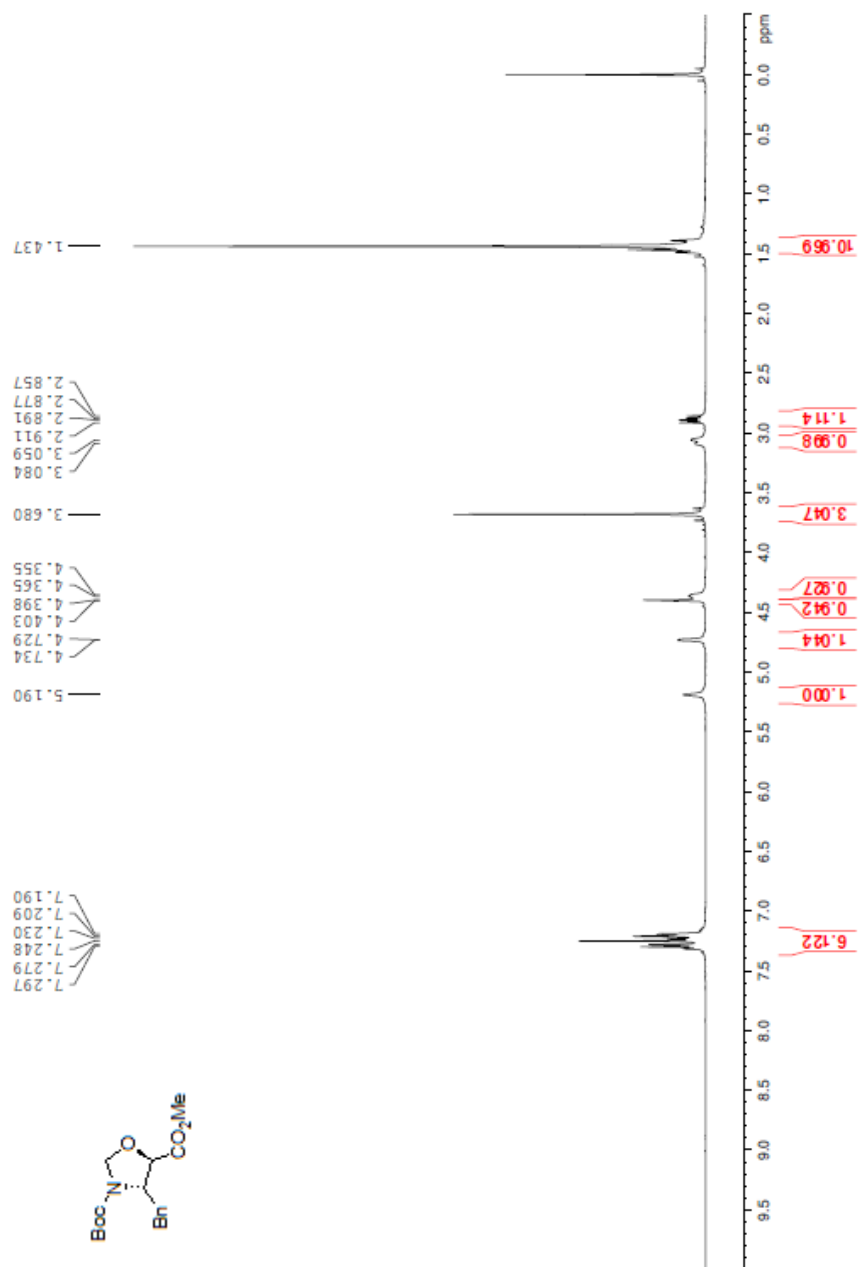
1. 600 MHz ¹ H-NMR spectrum (CDCl ₃ , 40 °C) of A22-Phe	136
2. 600 MHz ¹ H-NMR spectrum (CDCl ₃ , 40 °C) of A22-Leu	137
3. 400 MHz ¹ H-NMR spectrum (CDCl ₃ , 50 °C) of A23-Phe	138
4. 400 MHz ¹ H-NMR spectrum (CDCl ₃ , 50 °C) of A23-Leu	139
5. 400 MHz ¹ H-NMR spectrum (CDCl ₃ , 50 °C) of A23-Val	140
6. 400 MHz ¹ H-NMR spectrum (CDCl ₃ , 50 °C) of A23-Ala	141
7. 400 MHz ¹ H-NMR spectrum (CDCl ₃ , 50 °C) of A23-Ser	142
8. 400 MHz ¹ H-NMR spectrum (CDCl ₃ , 50 °C) of A24-Phe	143
9. 400 MHz ¹ H-NMR spectrum (CDCl ₃ , 50 °C) of A24-Leu	144
10. 400 MHz ¹ H-NMR spectrum (DMSO- <i>d</i> ₆) of (2 <i>S</i> ,3 <i>R</i>)-AHPBA	145
11. 400 MHz ¹ H-NMR spectrum (MeOH- <i>d</i> ₄) of (2 <i>S</i> ,3 <i>R</i>)-AHMHA ...	146
12. 400 MHz ¹ H-NMR spectrum (CDCl ₃) of <i>ent</i> - A25-Phe	147
13. 400 MHz ¹ H-NMR spectrum (CDCl ₃) of A28-Phe	148
14. 400 MHz ¹ H-NMR spectrum (CDCl ₃) of A28-Phe'-Val	149
15. 400 MHz ¹ H-NMR spectrum (CDCl ₃) of A28-Leu	150
16. 400 MHz ¹ H-NMR spectrum (CDCl ₃) of A28-Val	151
17. 400 MHz ¹ H-NMR spectrum (MeOH- <i>d</i> ₄) of A29	152
18. 400 MHz ¹ H-NMR spectrum (MeOH- <i>d</i> ₄) of A30	153
19. 400 MHz ¹ H-NMR spectrum (CDCl ₃) of A31	154
20. 400 MHz ¹ H-NMR spectrum (CDCl ₃) of A32	155
21. 400 MHz ¹ H-NMR spectrum (CDCl ₃) of A33	156
22. 400 MHz ¹ H-NMR spectrum (DCI) of L-TBOA	157

23. 400 MHz ^1H -NMR spectrum (CDCl_3) of <i>ent</i> - A34	158
24. 400 MHz ^1H -NMR spectrum ($\text{DMSO}-d_6$) of A35	159
25. 400 MHz ^1H -NMR spectrum ($\text{DMSO}-d_6$) of <i>N</i> -(4-Iodophenyl)- acetamide.....	160
26. 400 MHz ^1H -NMR spectrum ($\text{DMSO}-d_6$) of <i>N</i> -(4-Bromophenyl)- acetamide.....	161
27. 400 MHz ^1H -NMR spectrum ($\text{DMSO}-d_6$) of <i>N</i> -(4-Chlorophenyl)- acetamide.....	162
28. 400 MHz ^1H -NMR spectrum ($\text{DMSO}-d_6$) of <i>N</i> -(4-Fluorophenyl)- acetamide.....	163
29. 400 MHz ^1H -NMR spectrum ($\text{DMSO}-d_6$) of <i>N</i> -(4-Nitrophenyl)- acetamide.....	164
30. 400 MHz ^1H -NMR spectrum ($\text{DMSO}-d_6$) of <i>N</i> -(4-Methoxyphenyl)- acetamide.....	165
31. 300 MHz ^1H -NMR spectrum ($\text{DMSO}-d_6$) of <i>N</i> -(4-Hydroxyphenyl)- acetamide.....	166

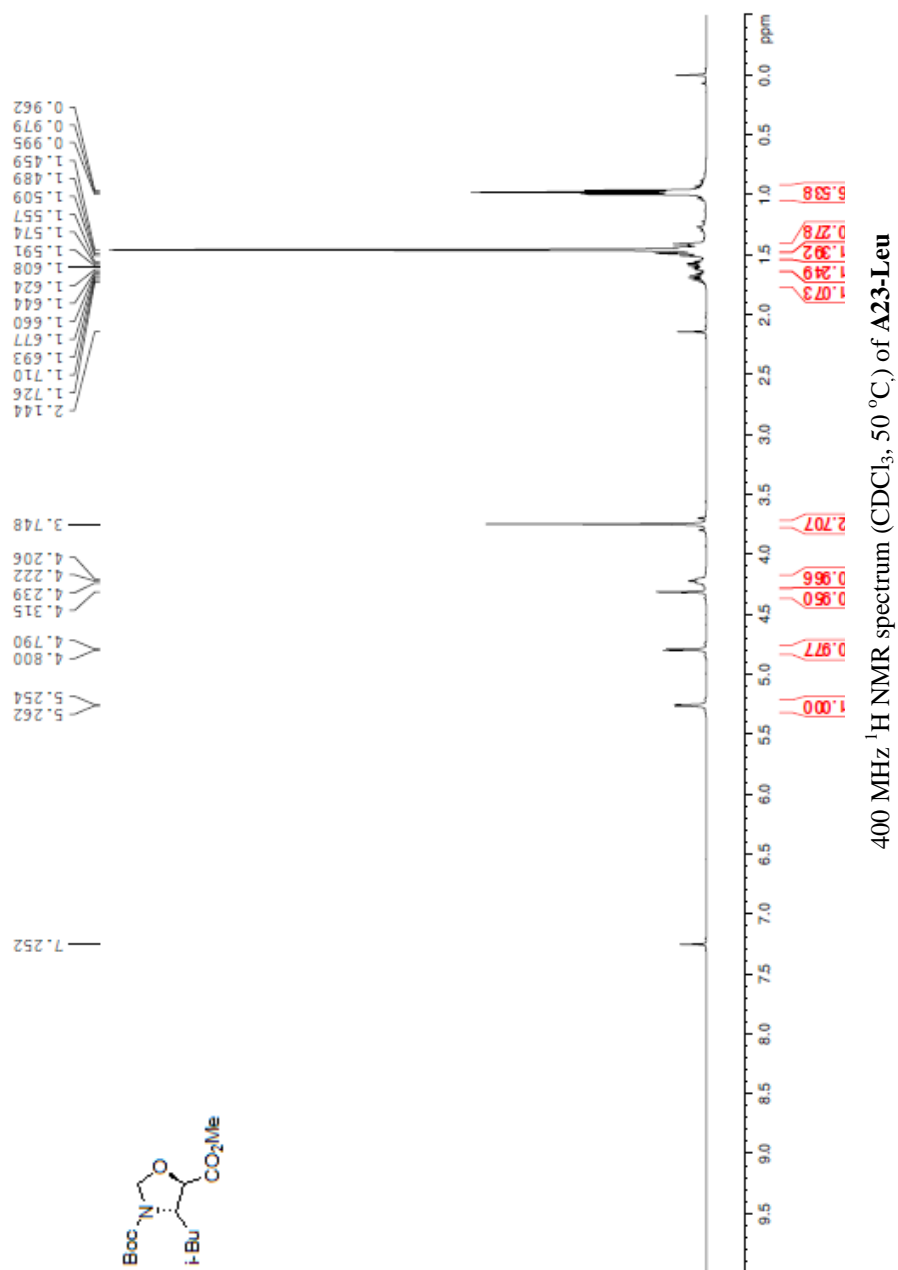


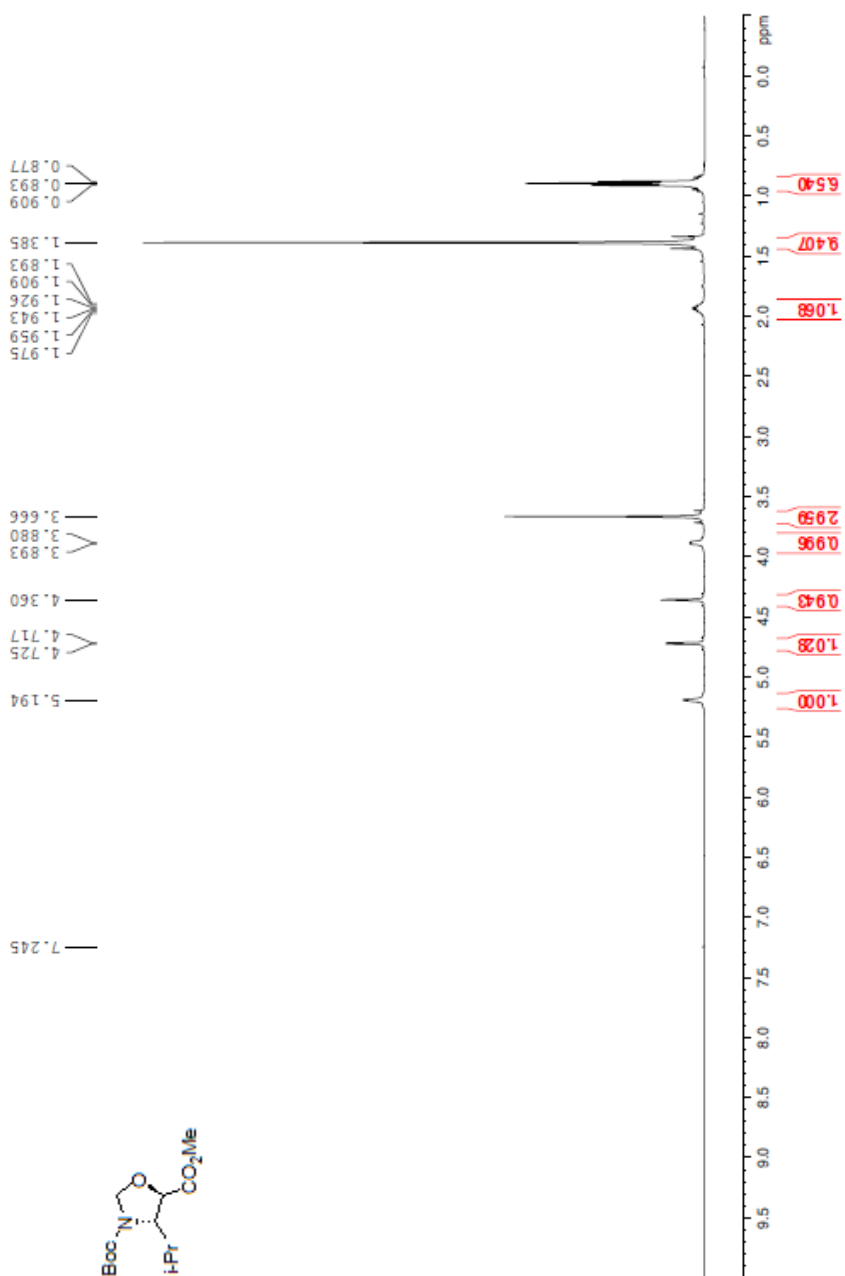


600 MHz ^1H NMR spectrum (CDCl_3 , 40 $^\circ\text{C}$) of A22-Leu

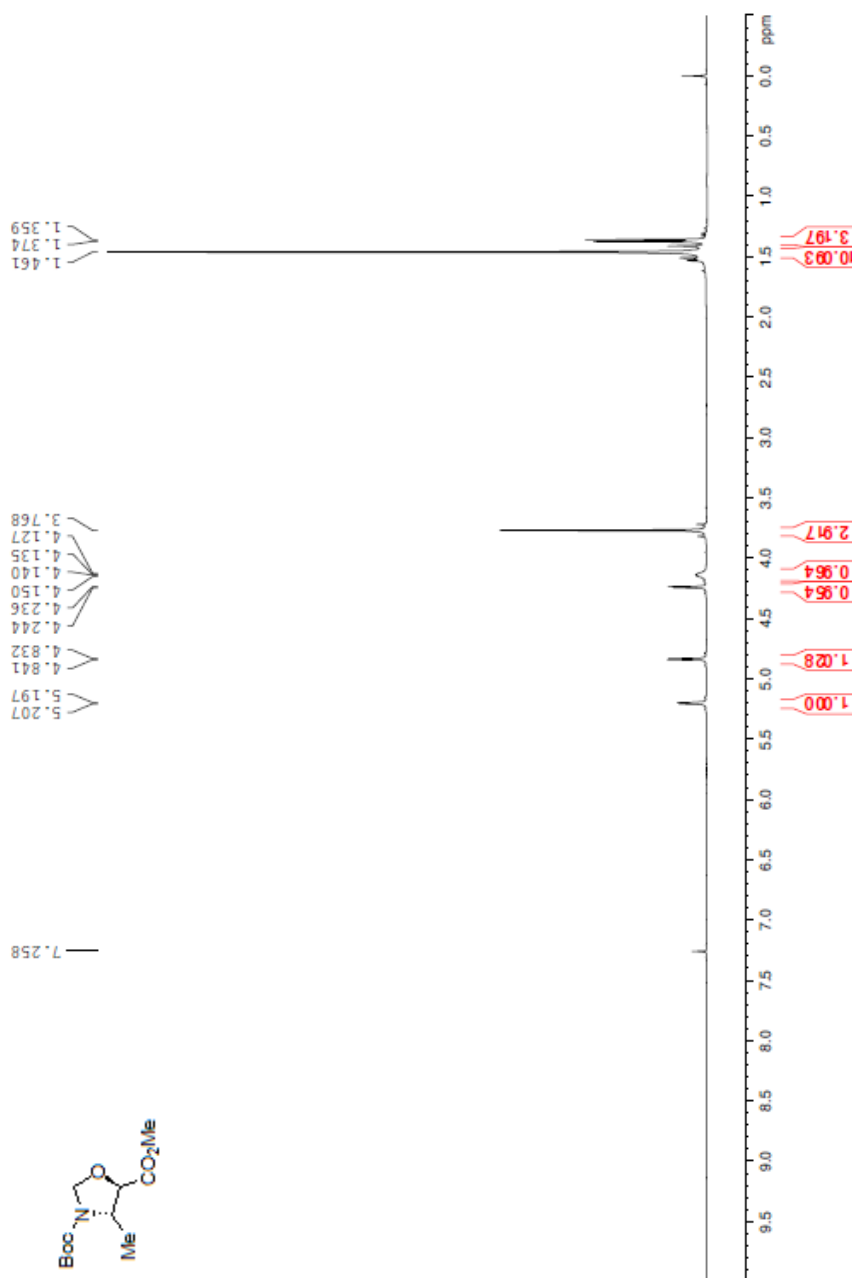


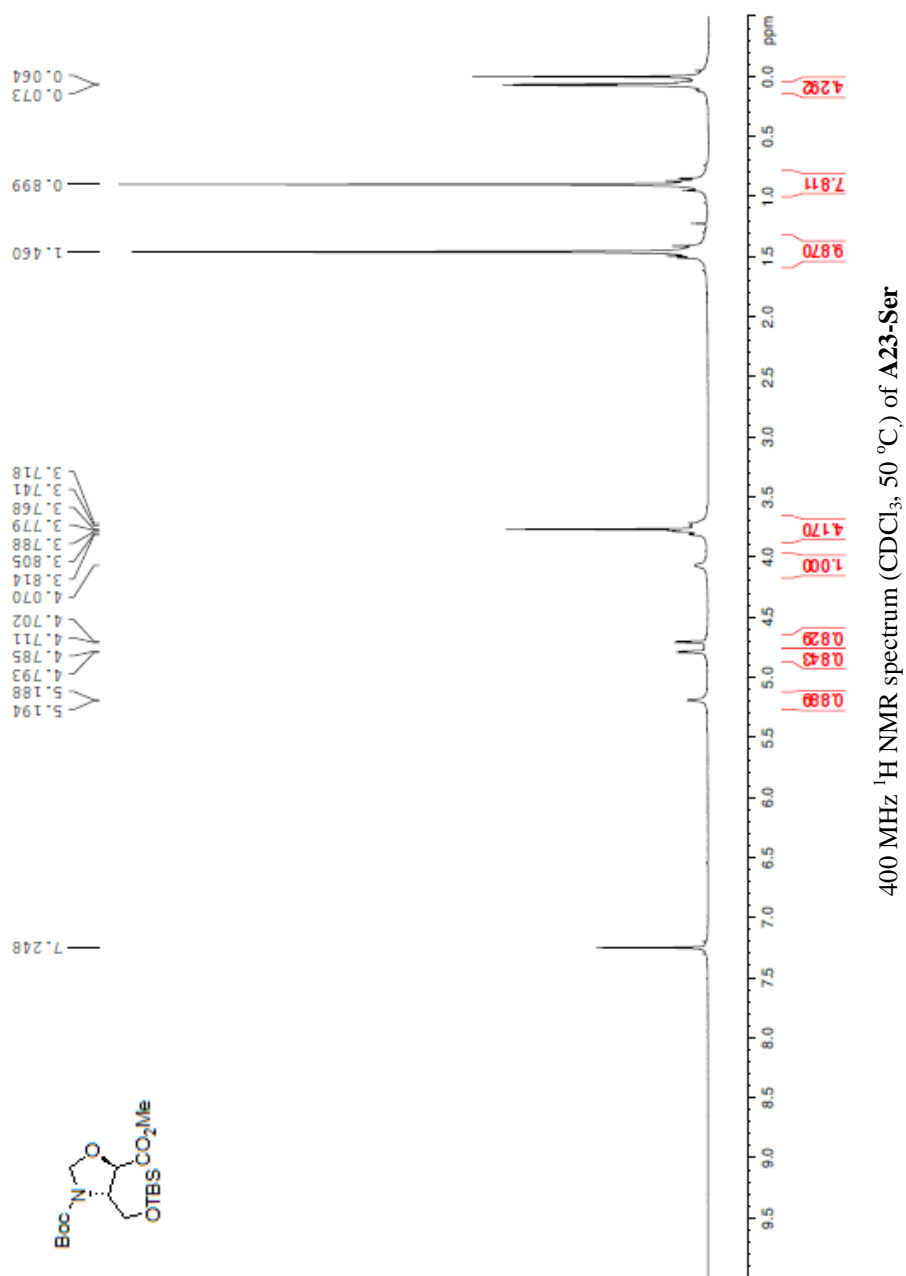
400 MHz ^1H NMR spectrum (CDCl_3 , 50 $^\circ\text{C}$) of A23-Phe

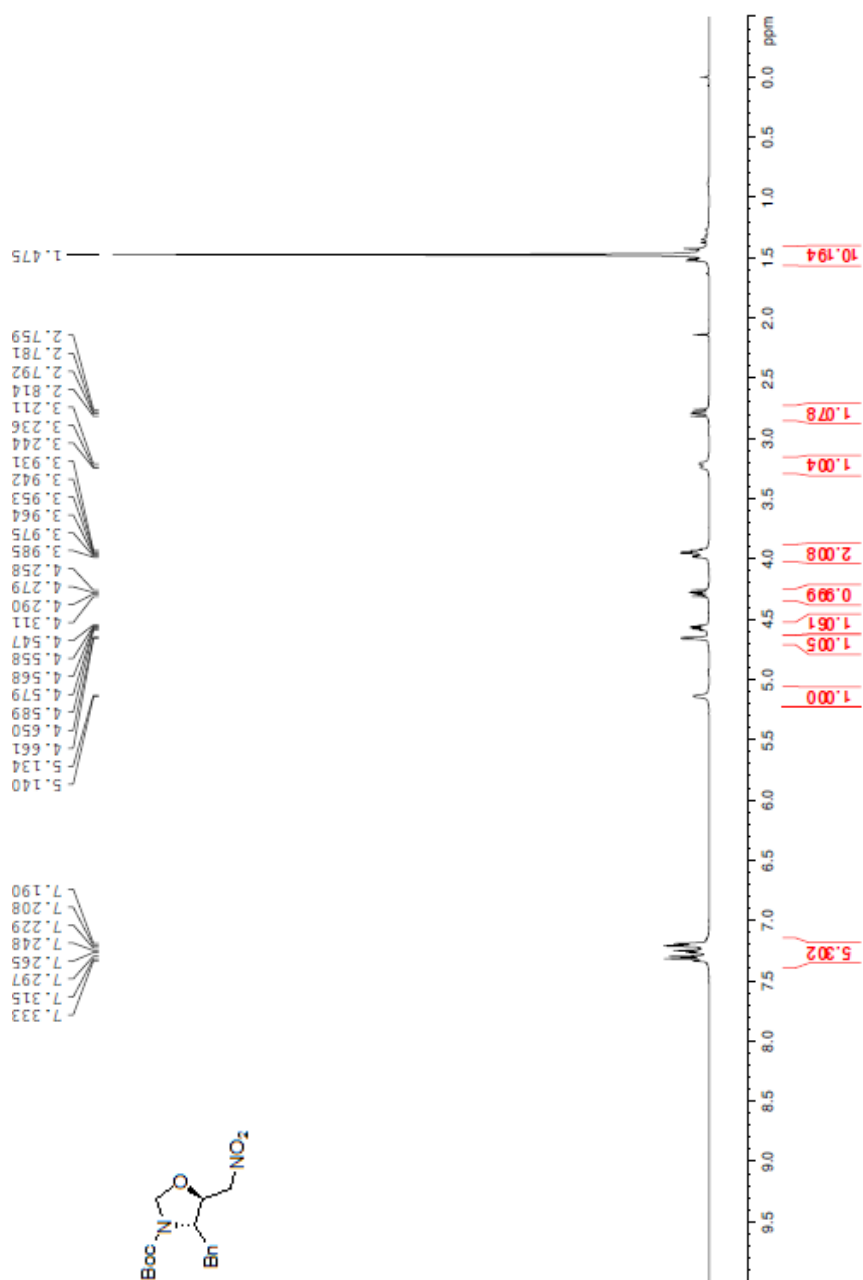




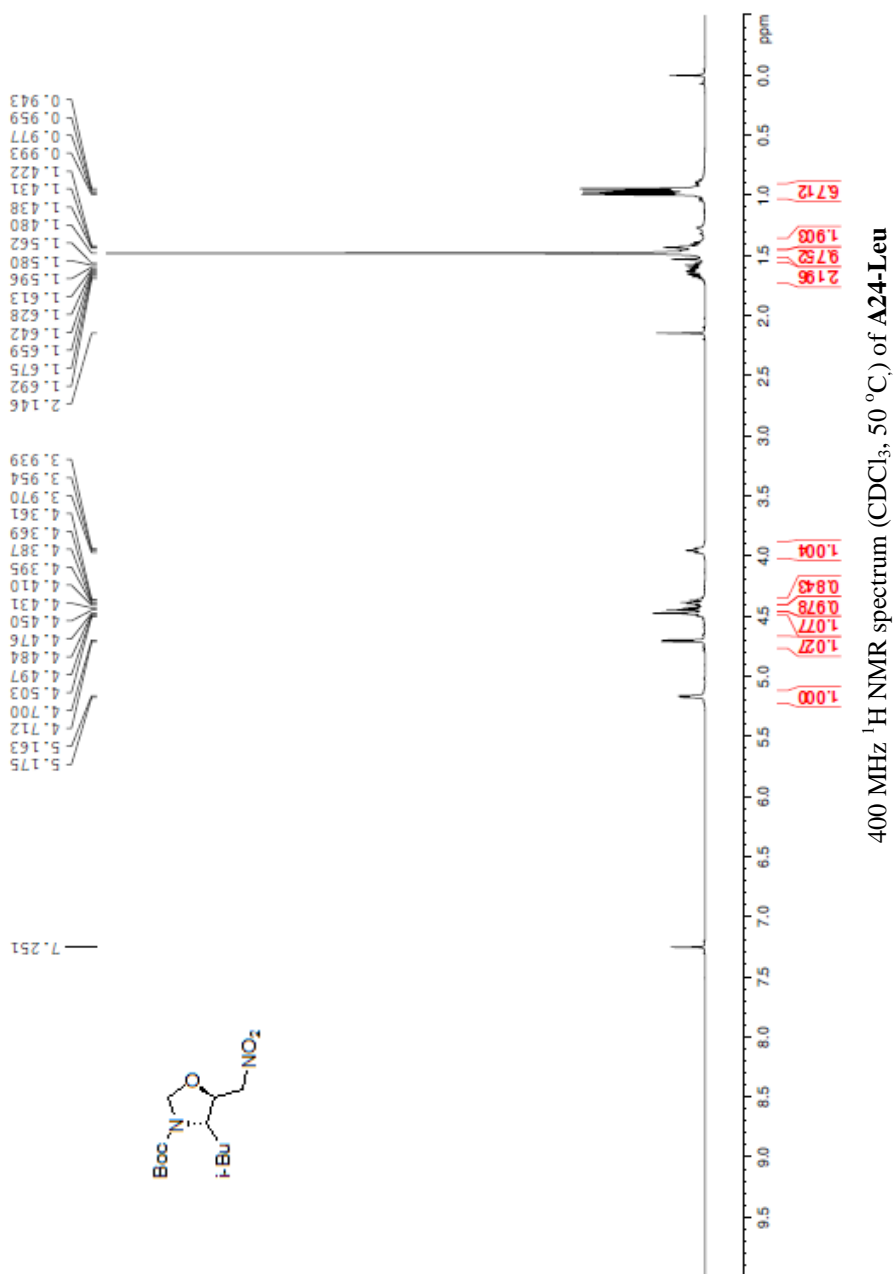
400 MHz ^1H NMR spectrum (CDCl_3 , 50 $^\circ\text{C}$) of A23-Val

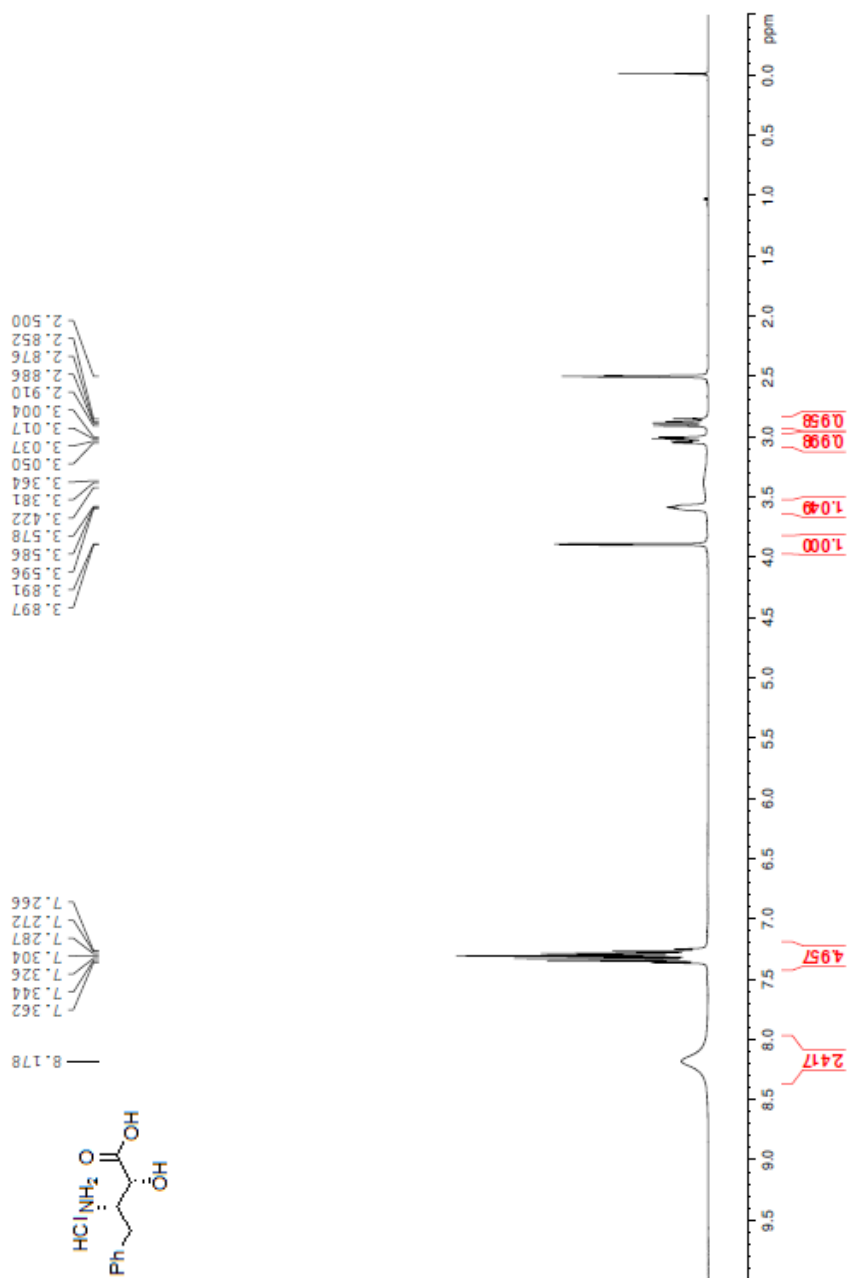




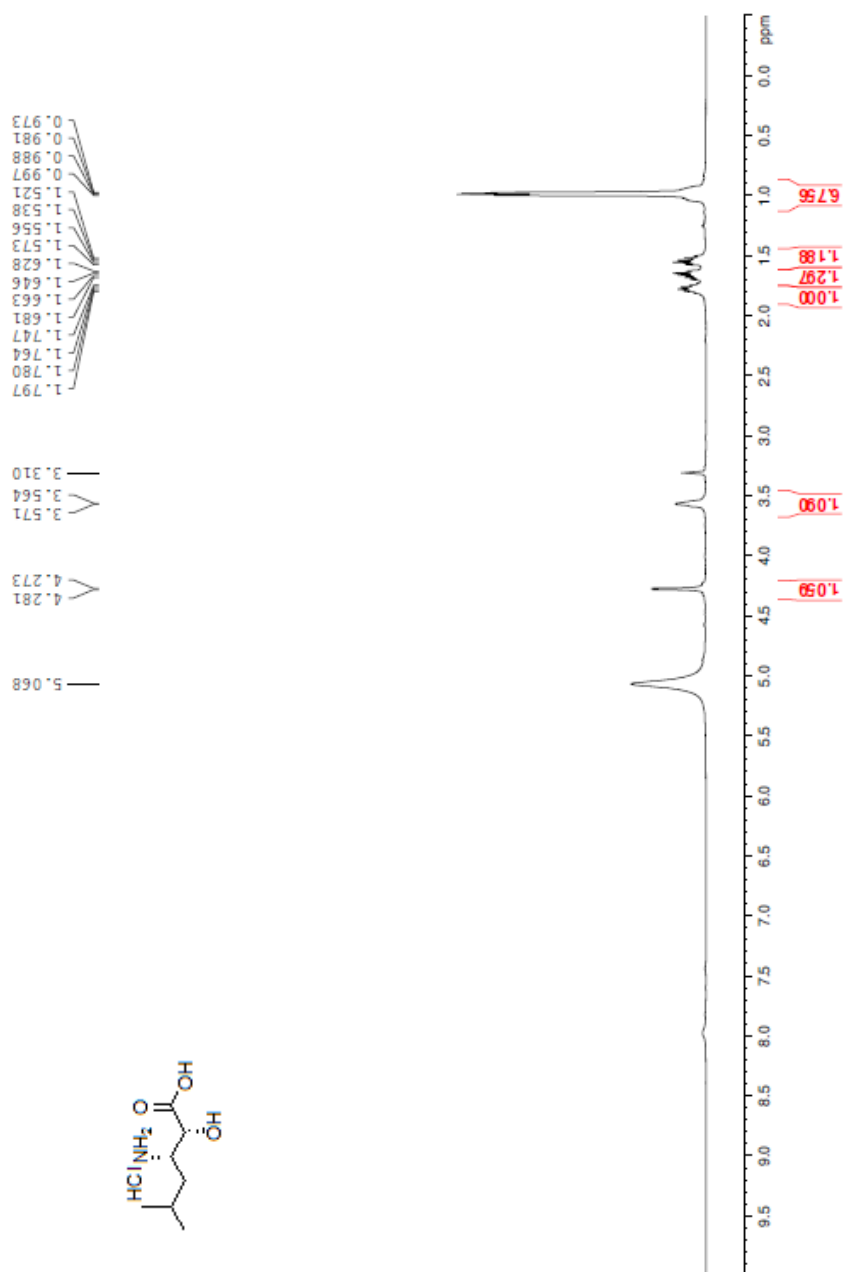


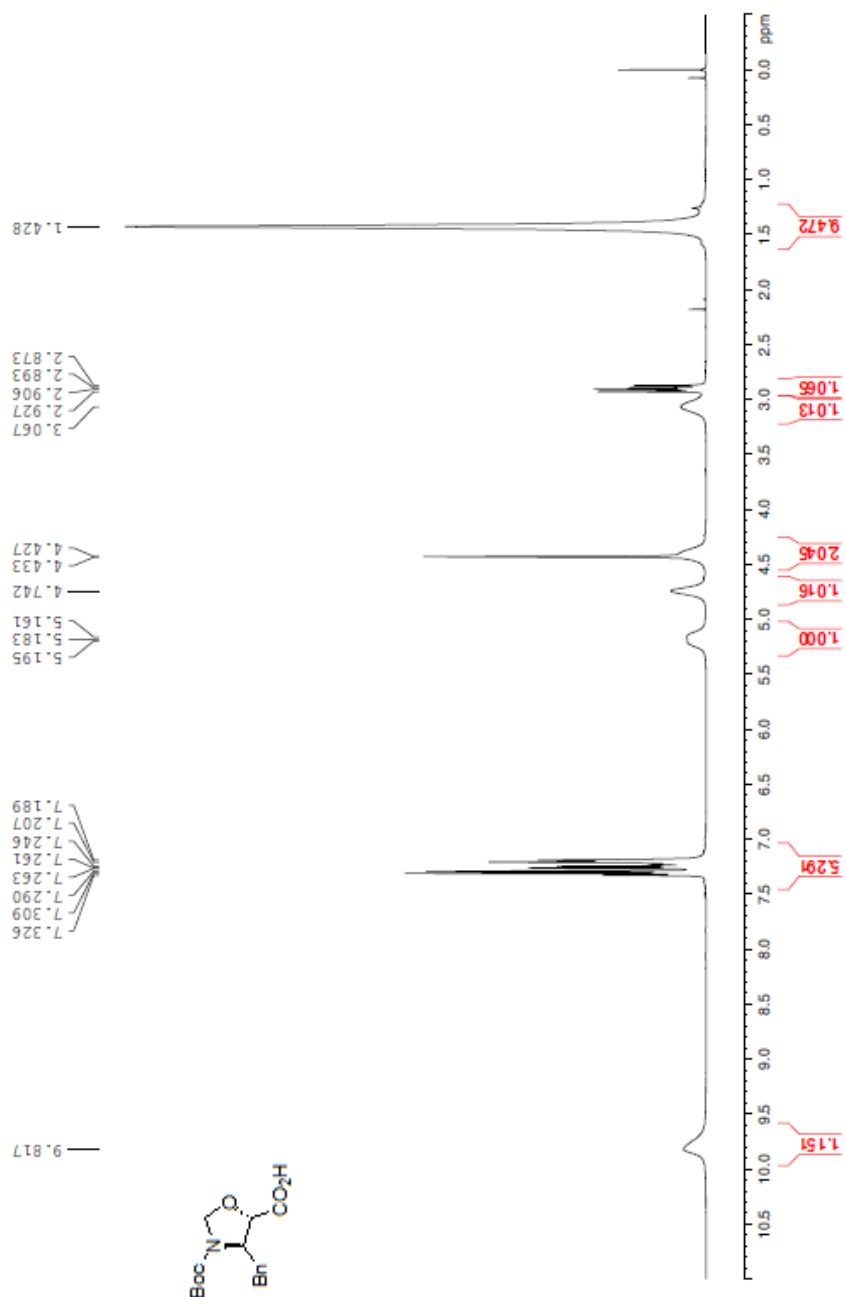
400 MHz ^1H NMR spectrum (CDCl_3 , 50 $^\circ\text{C}$) of A24-Phe

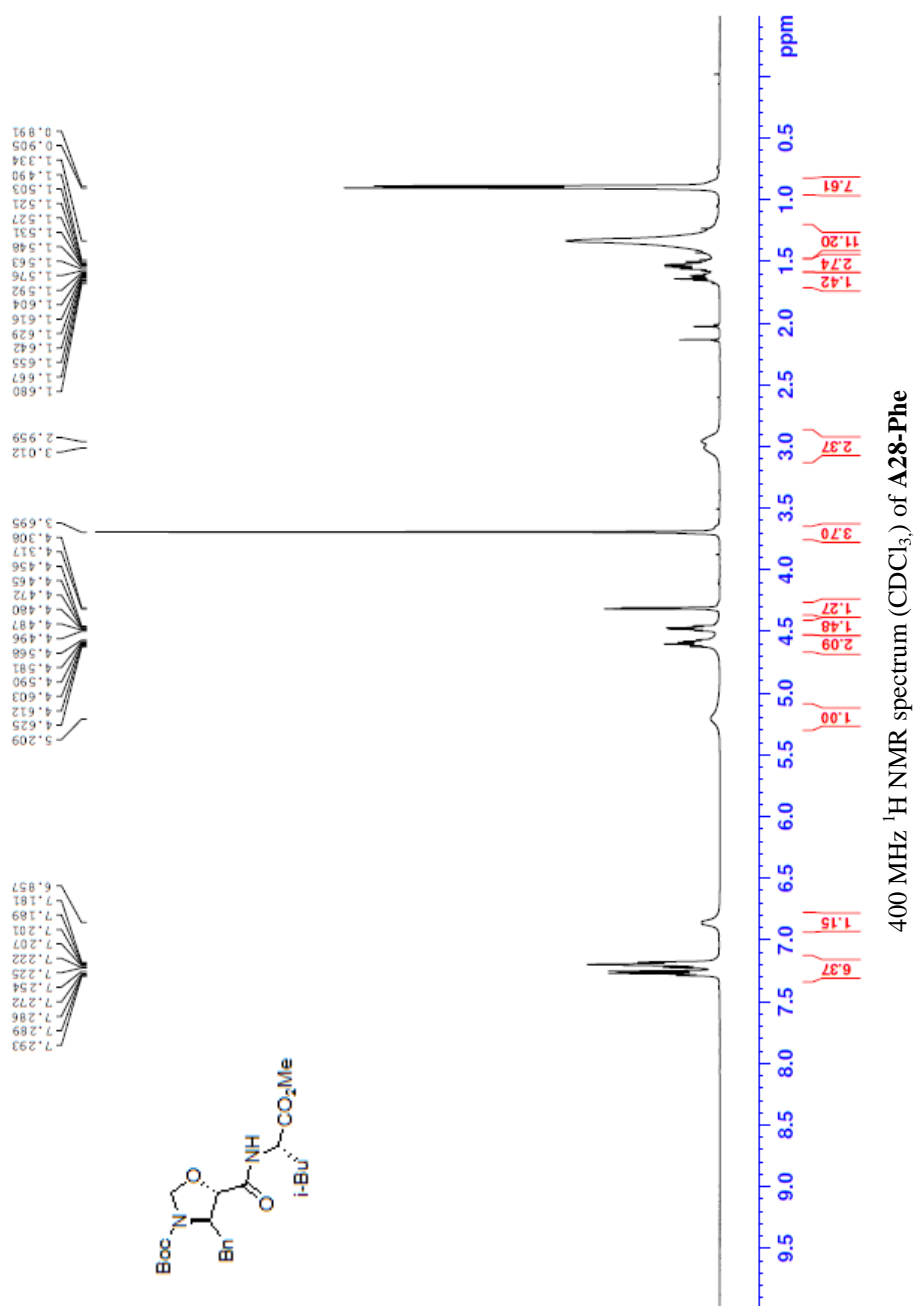


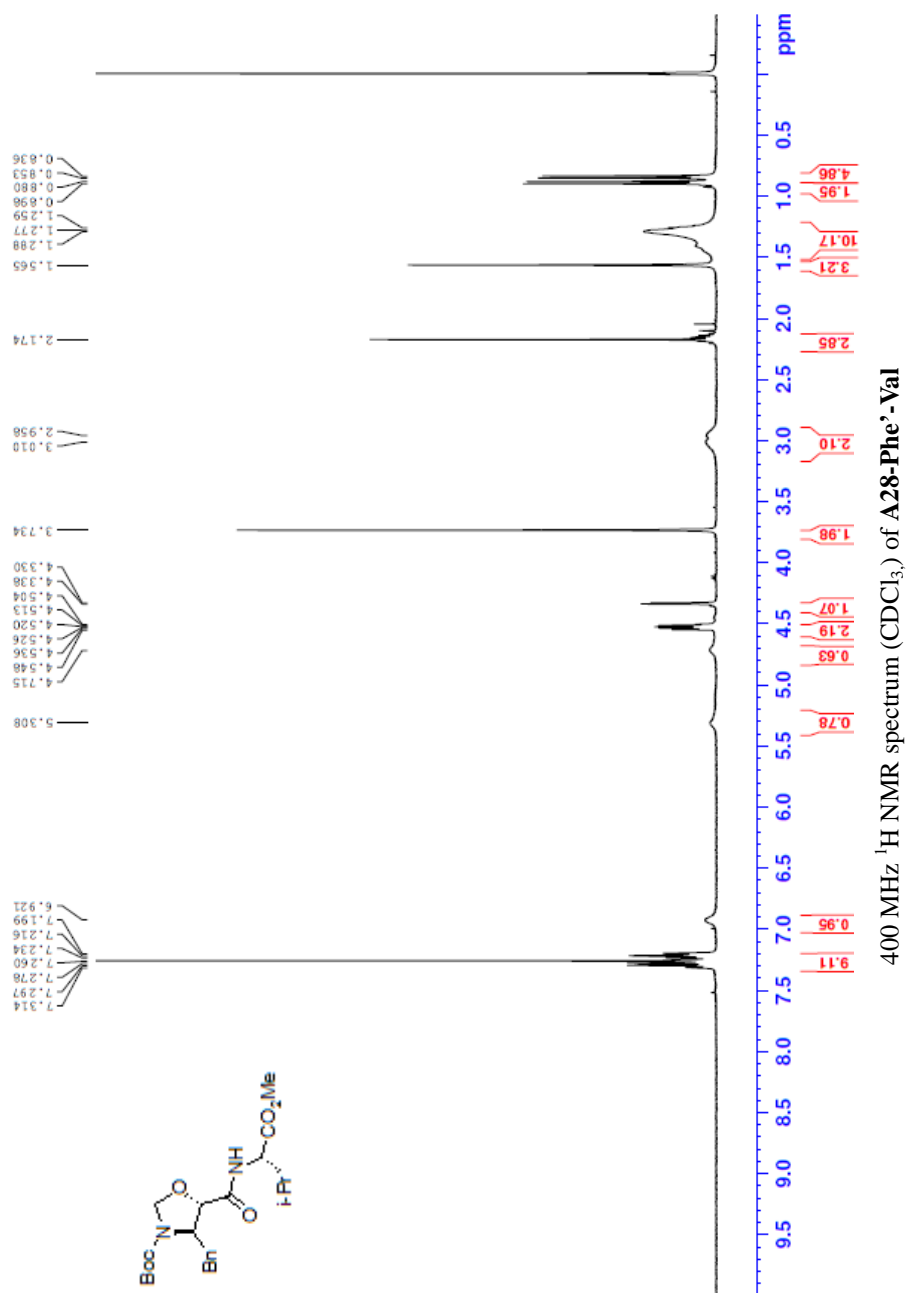


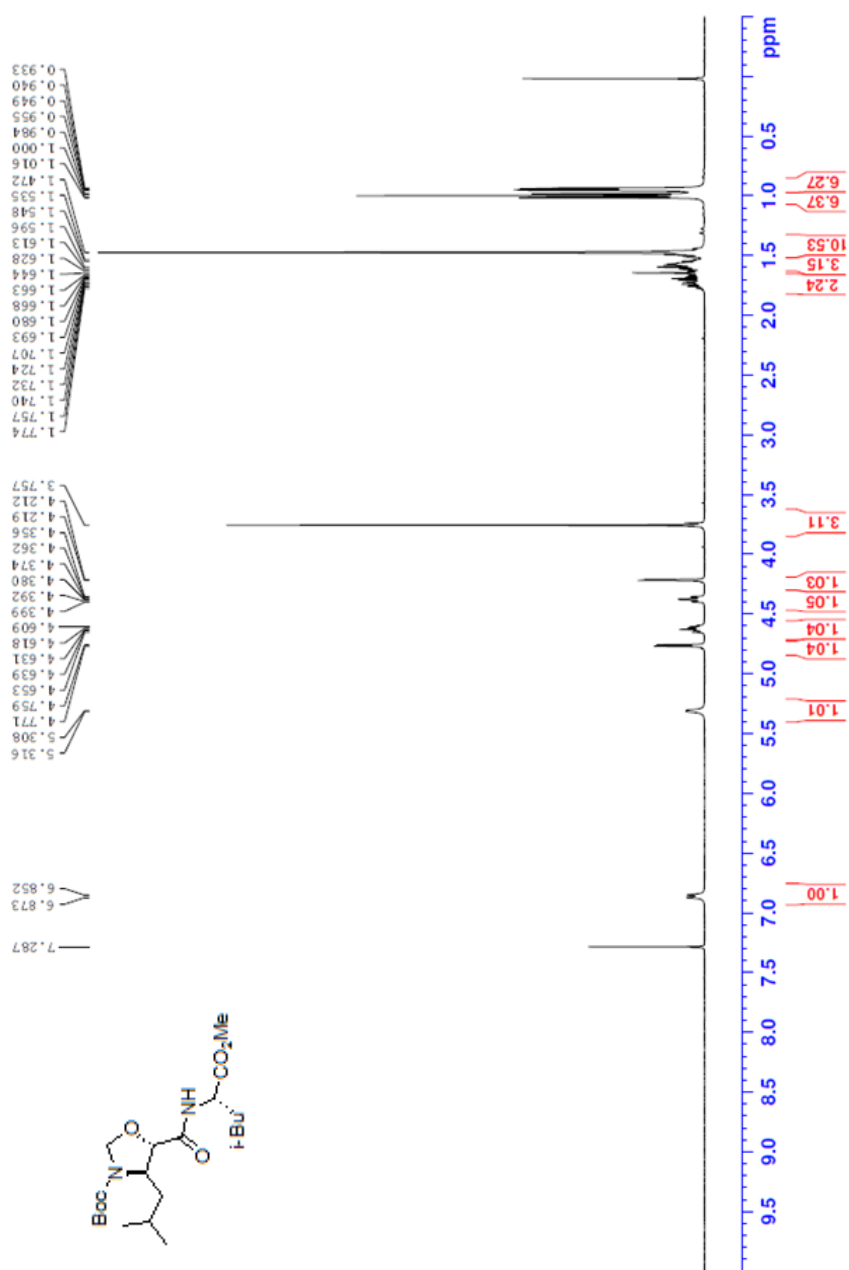
400 MHz ¹H NMR spectrum (DMSO-*d*₆) of (2*R*,3*S*)-AHPBA·HCl

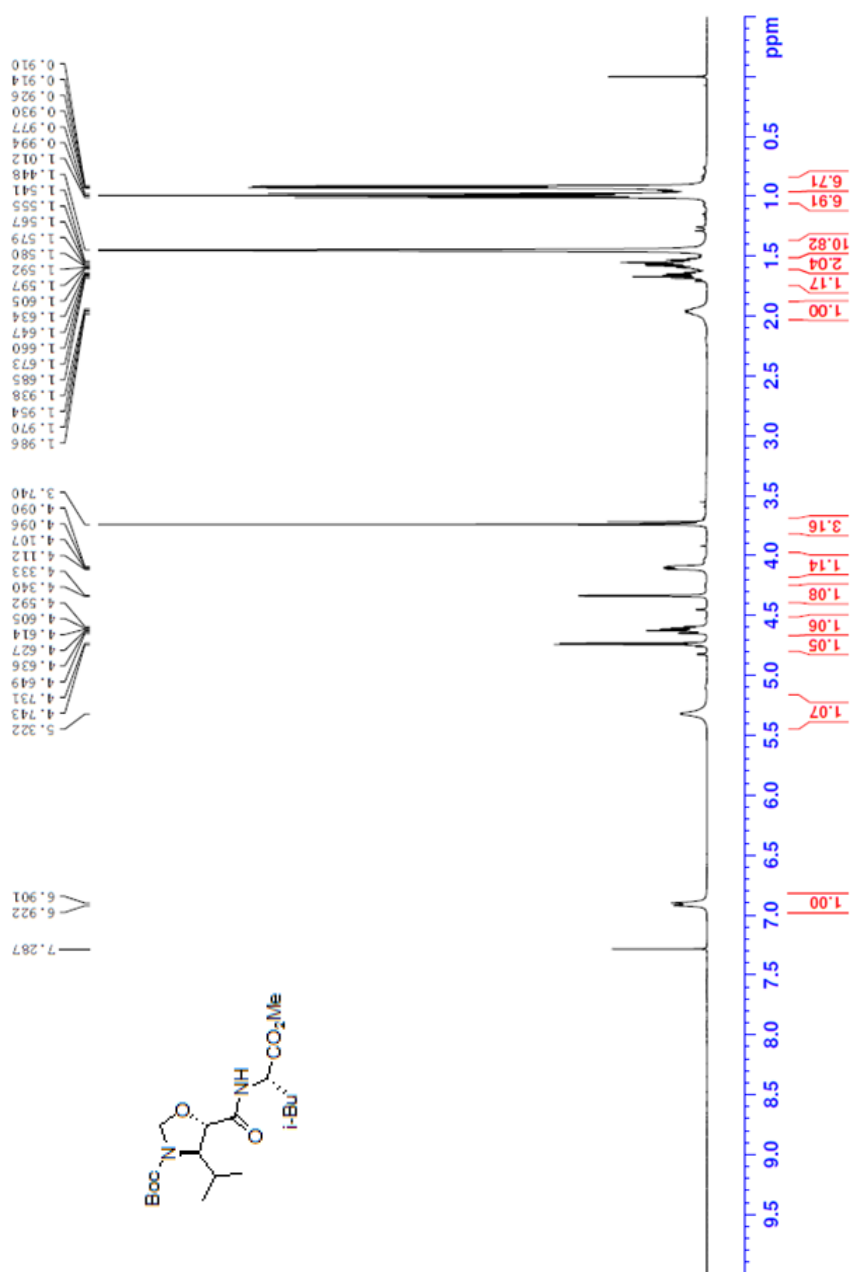




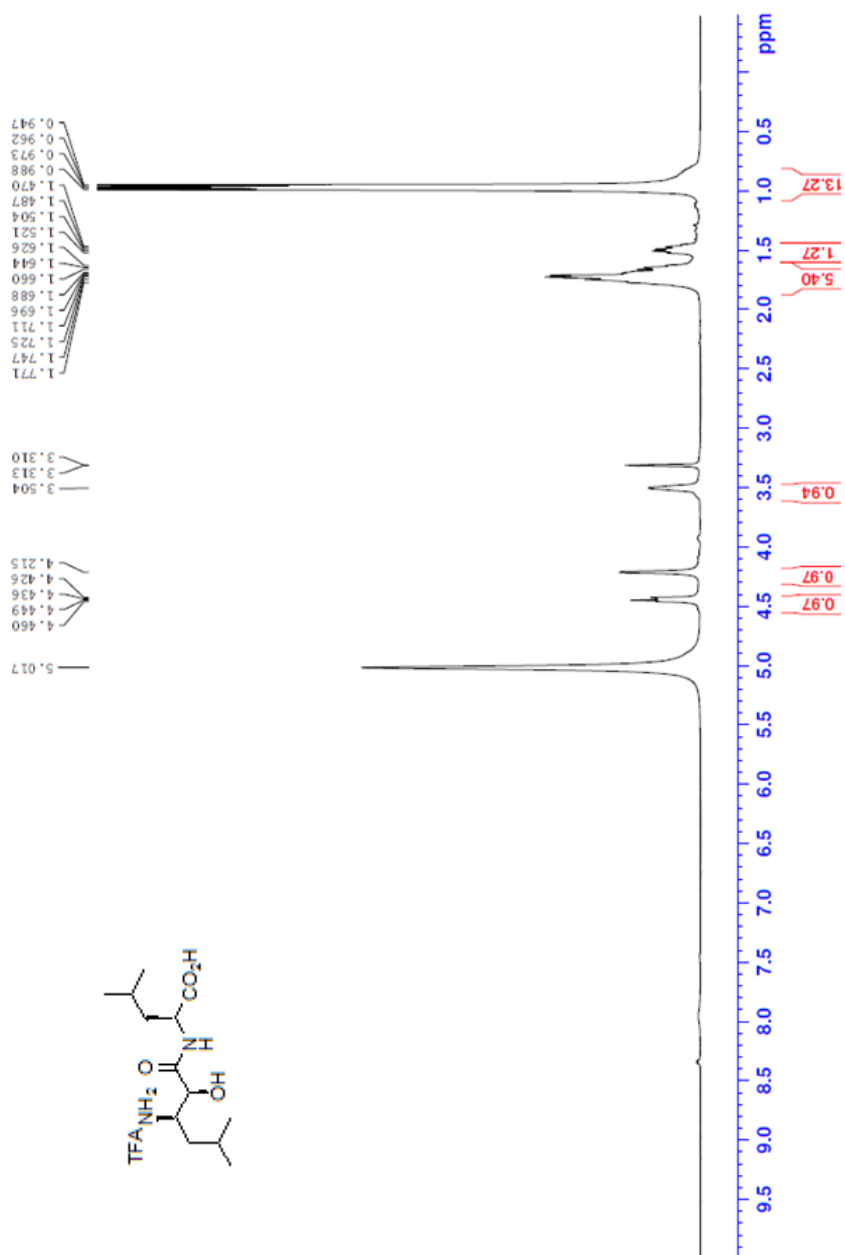


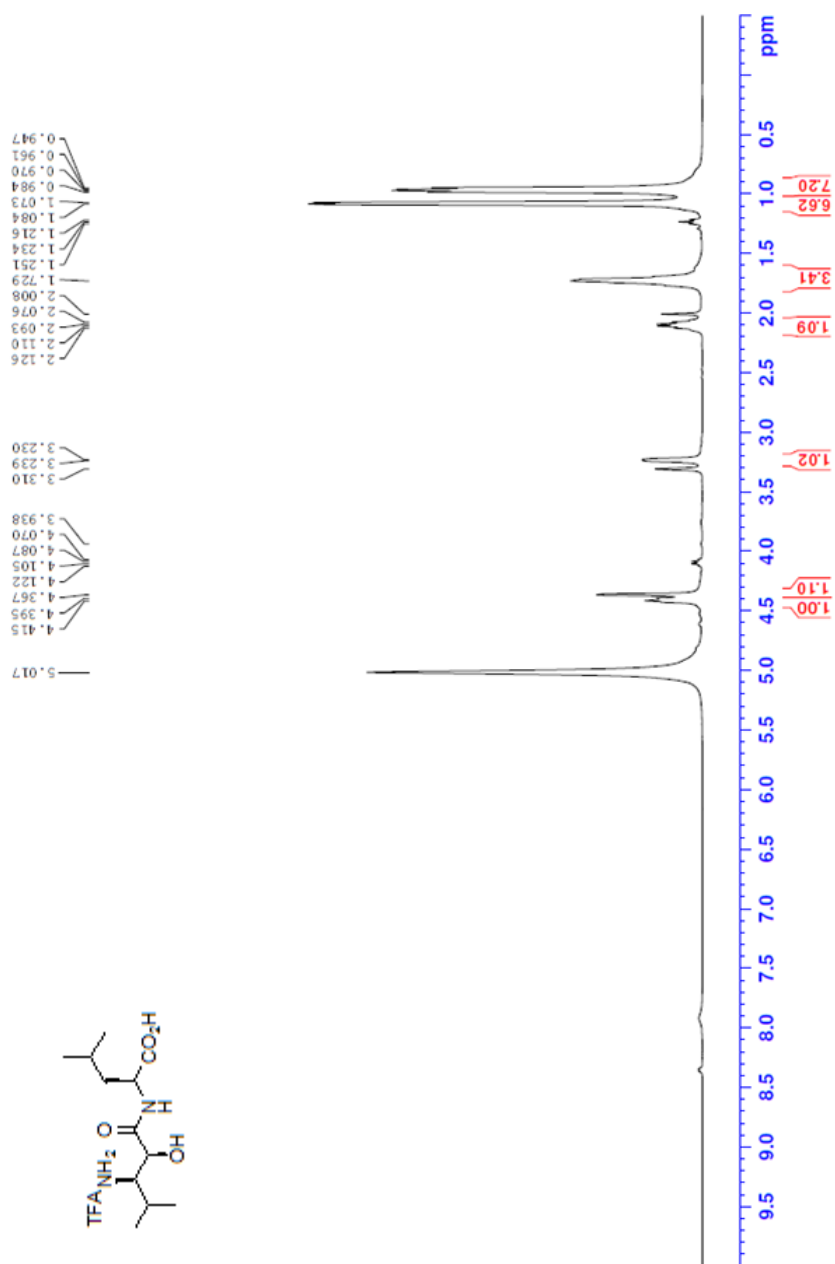


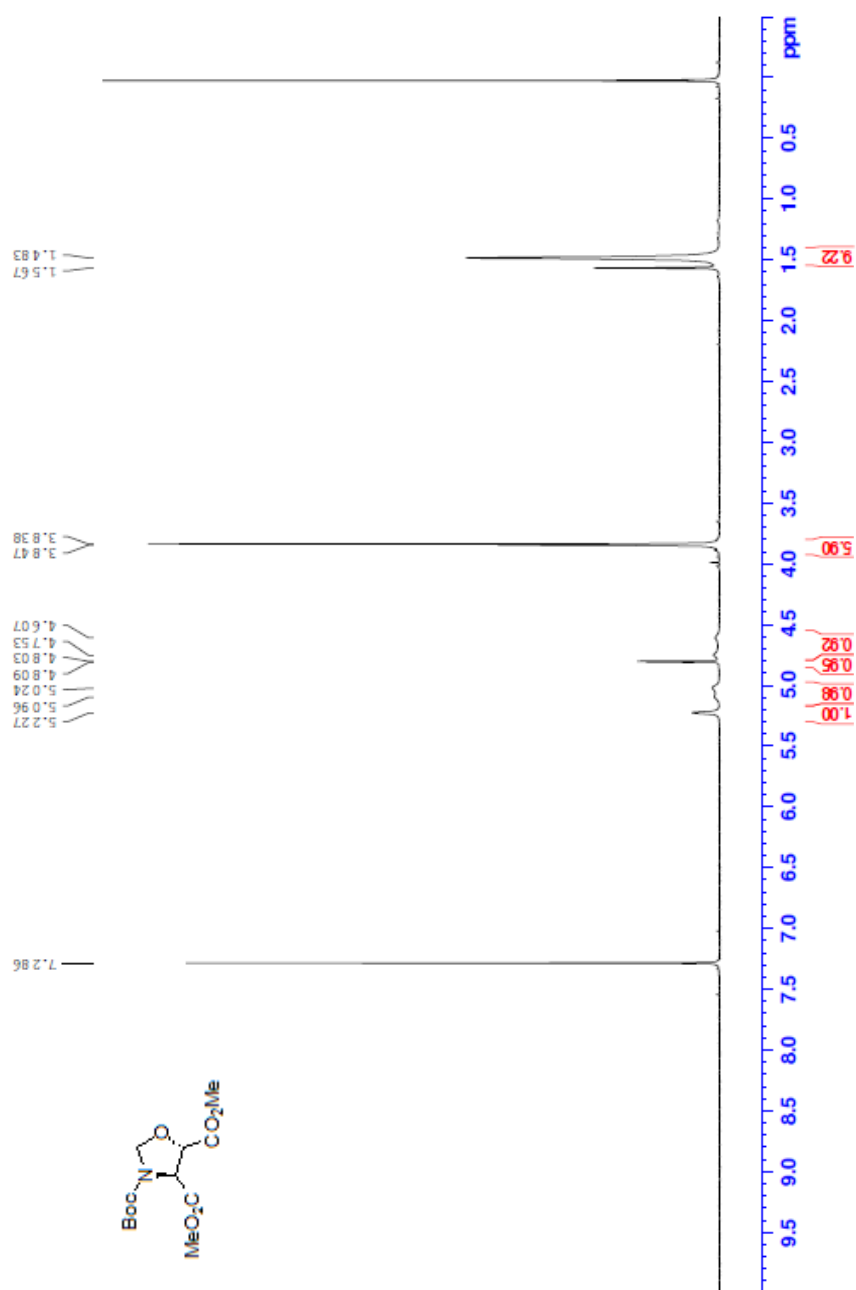


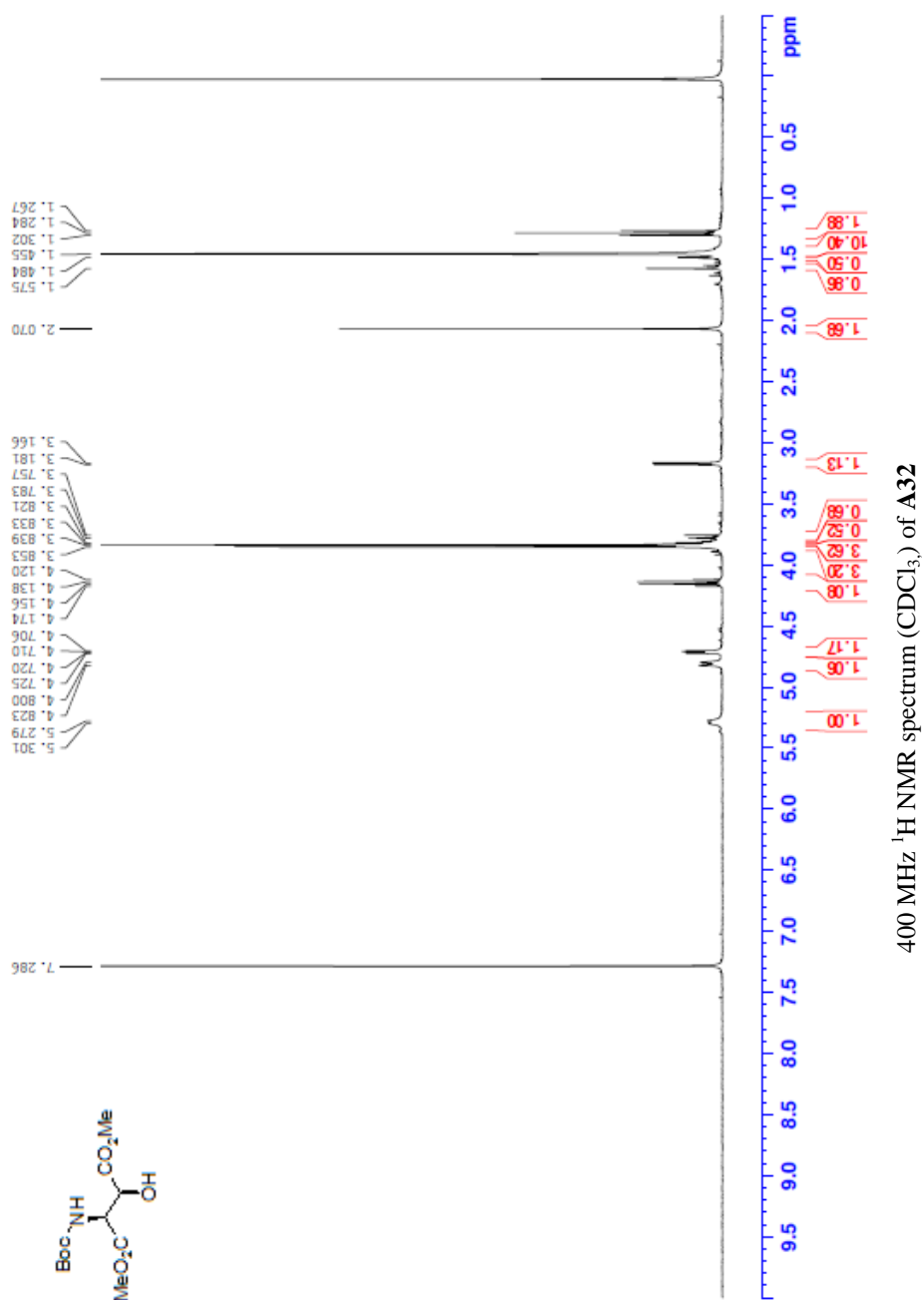


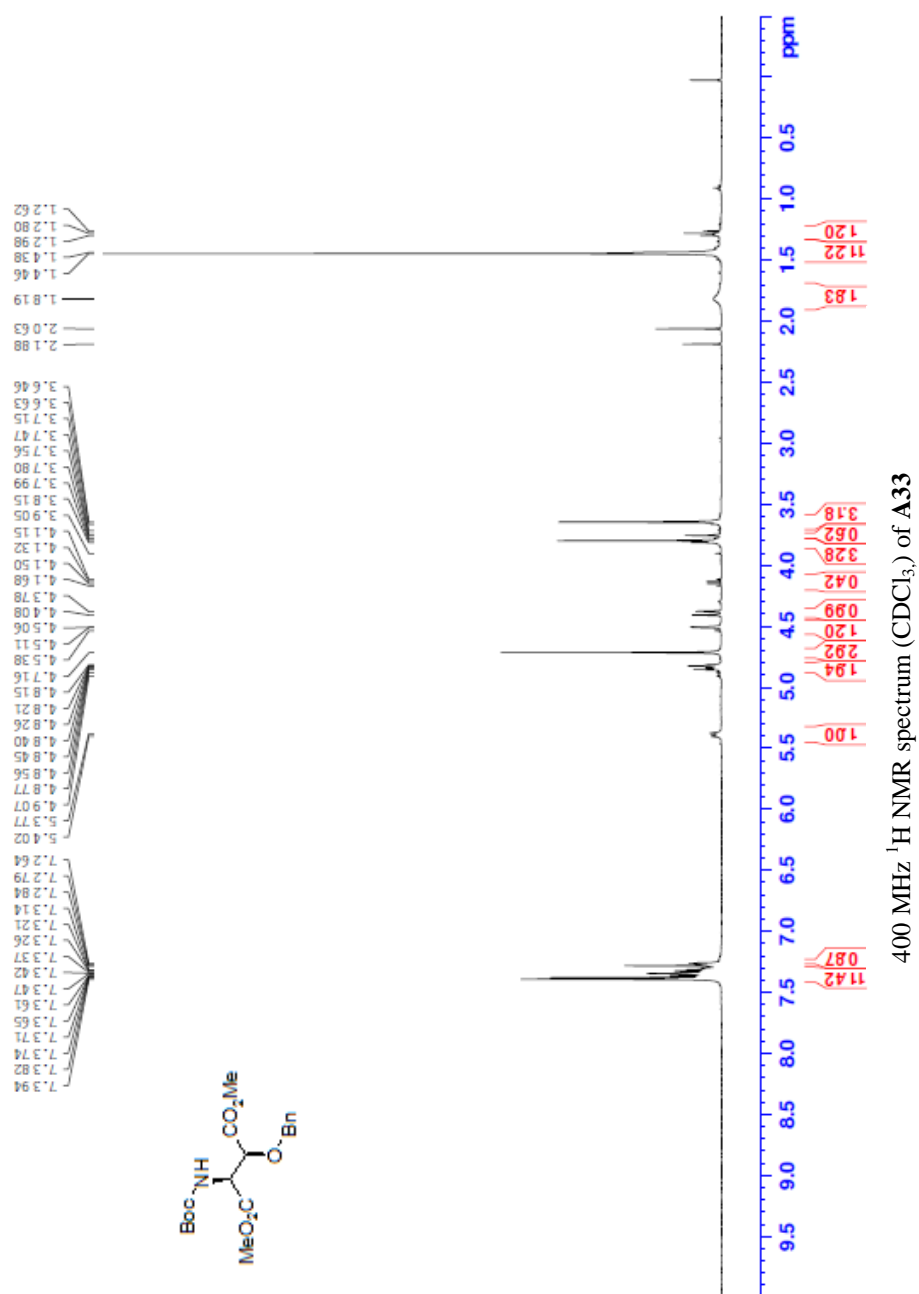
400 MHz ¹H NMR spectrum (CDCl₃) of A28-Val

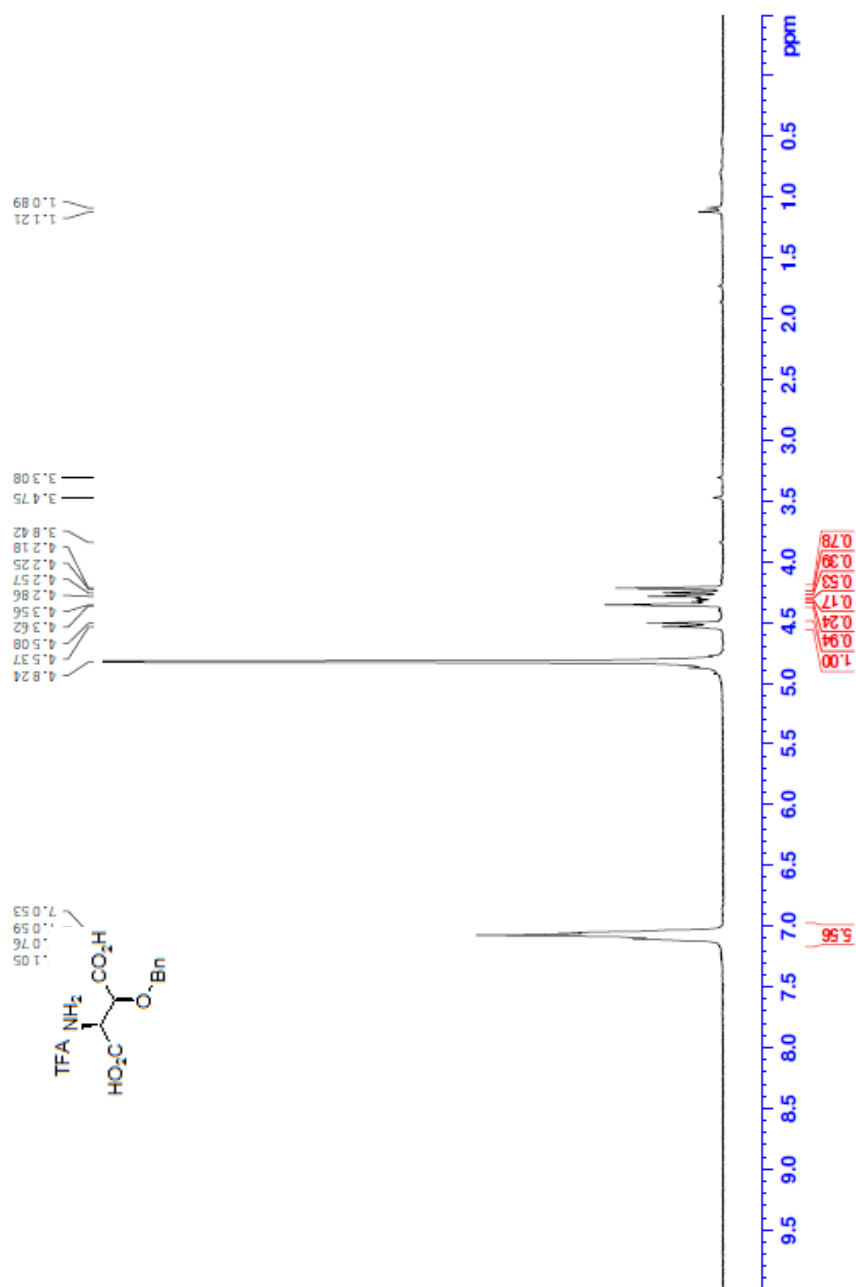




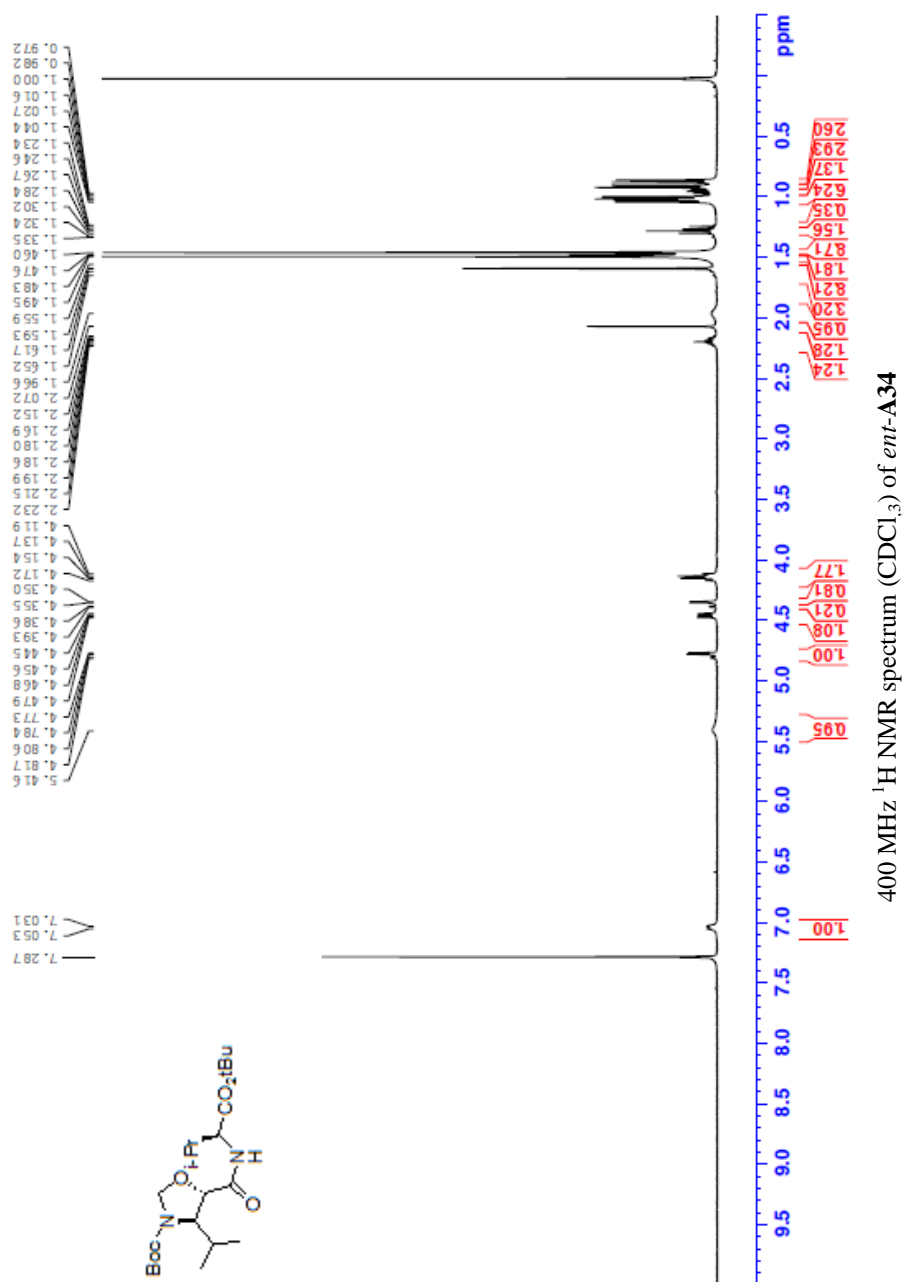


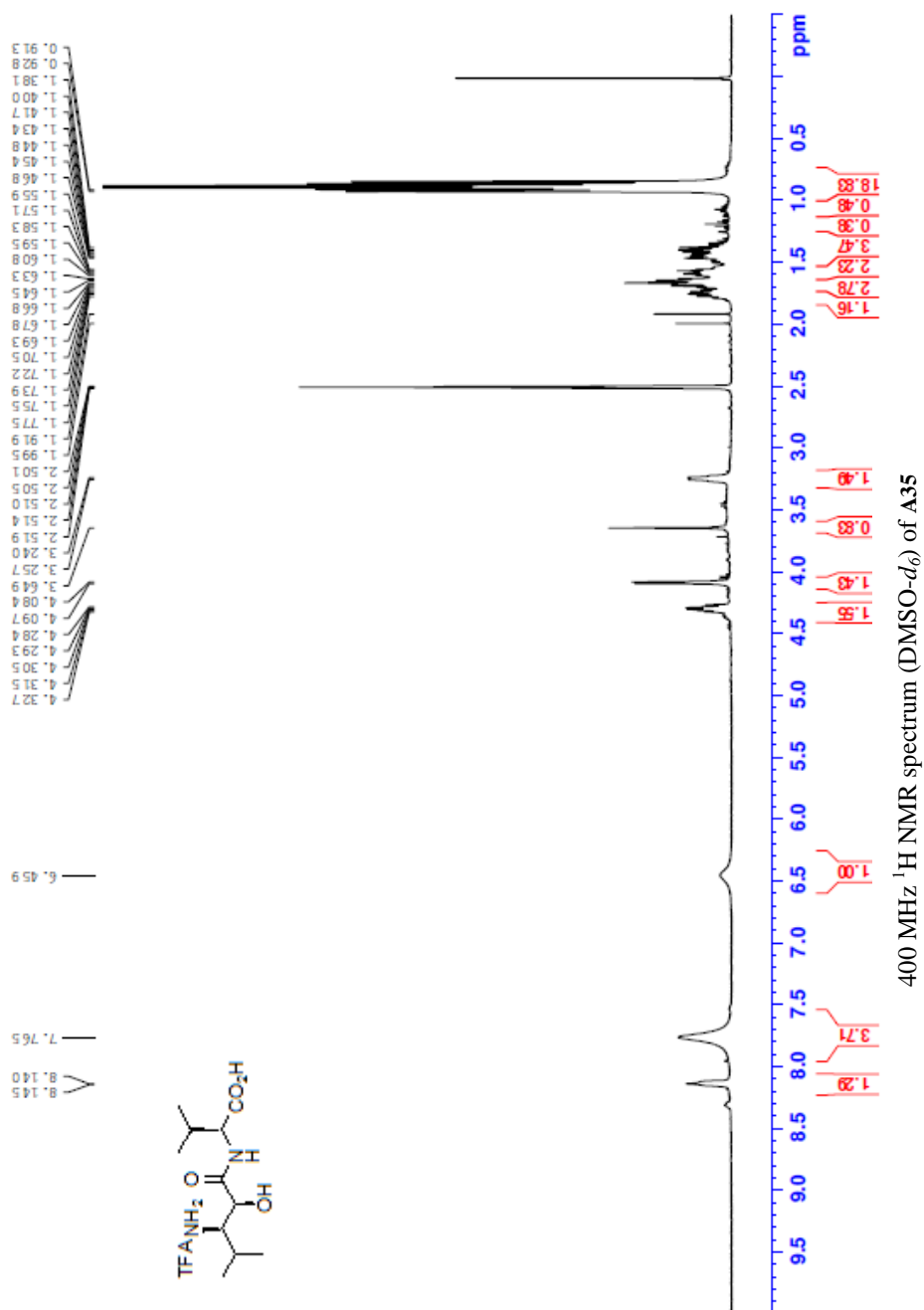


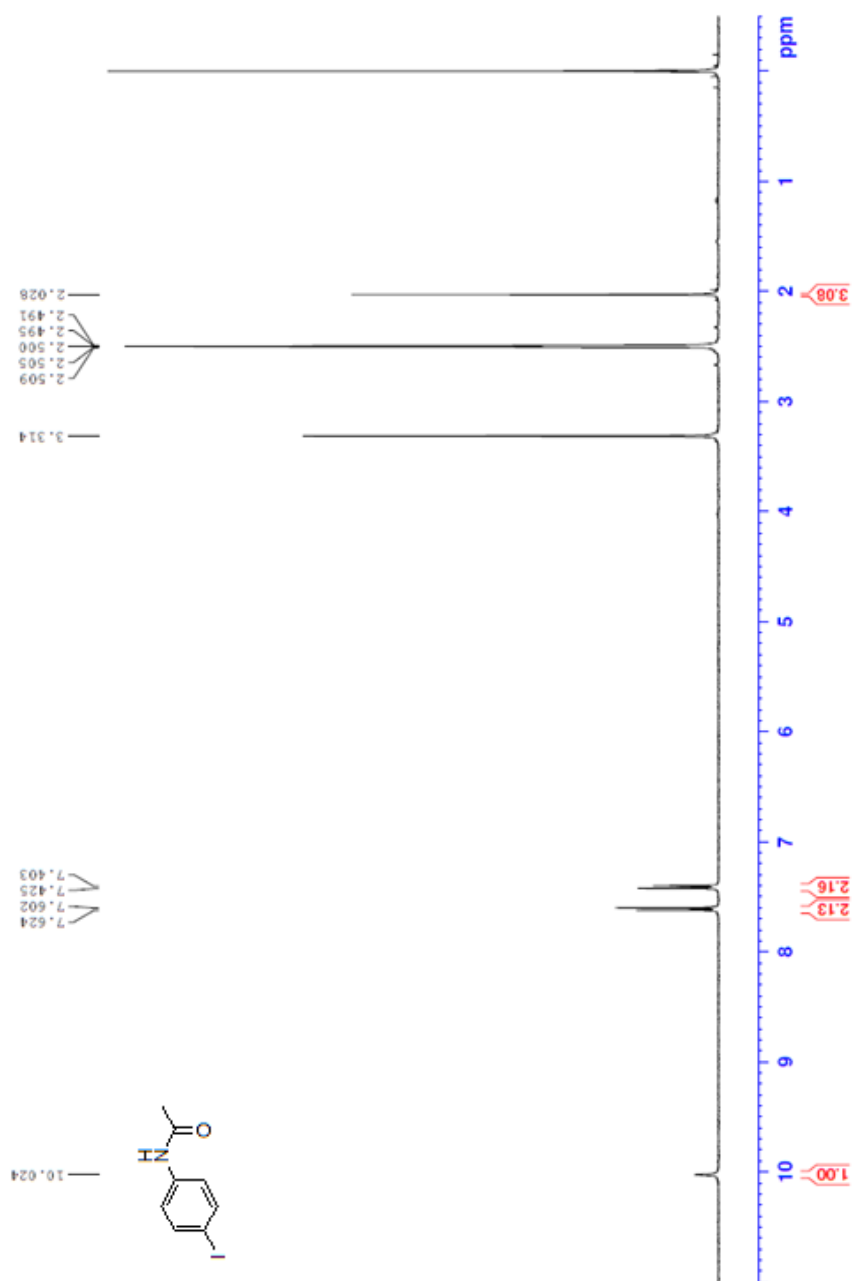




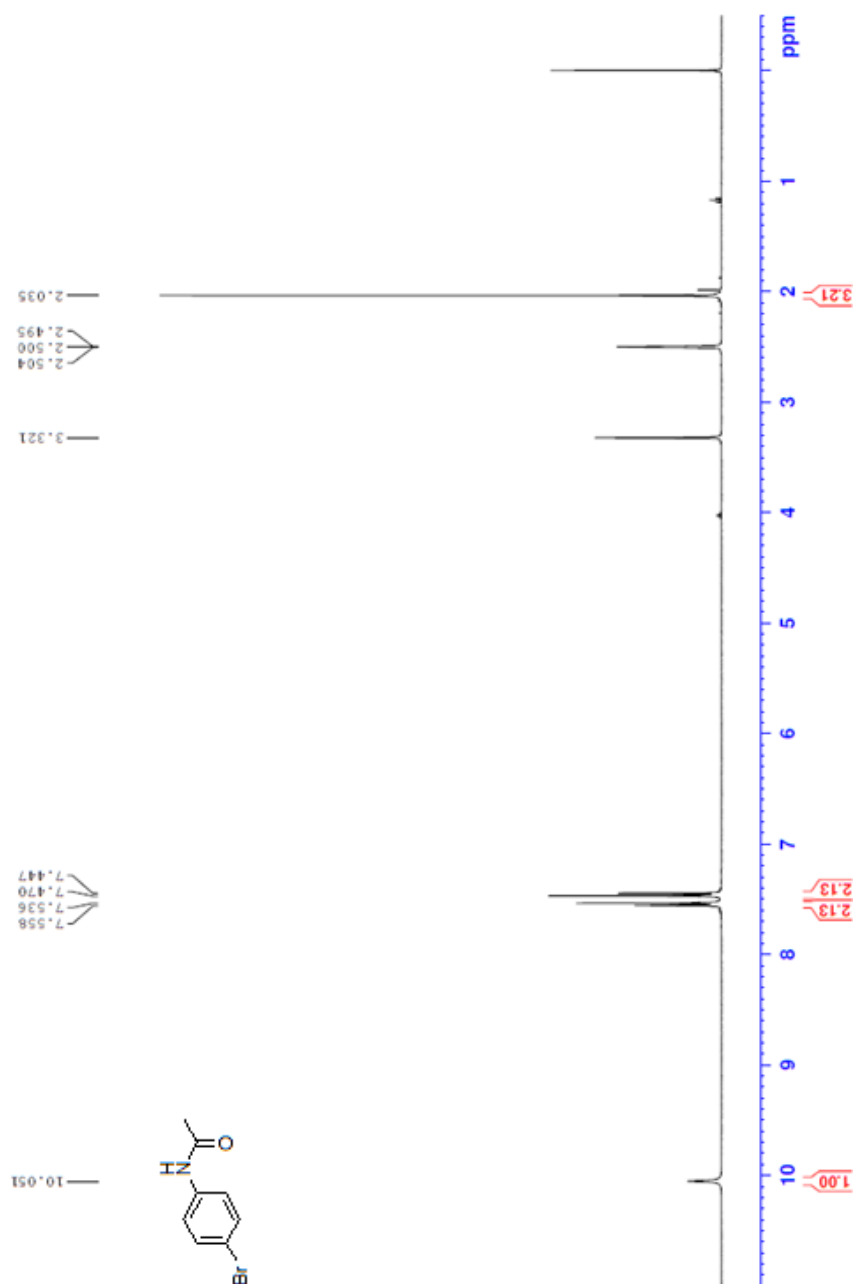
400 MHz ^1H NMR spectrum (DCl) of L-TBOA

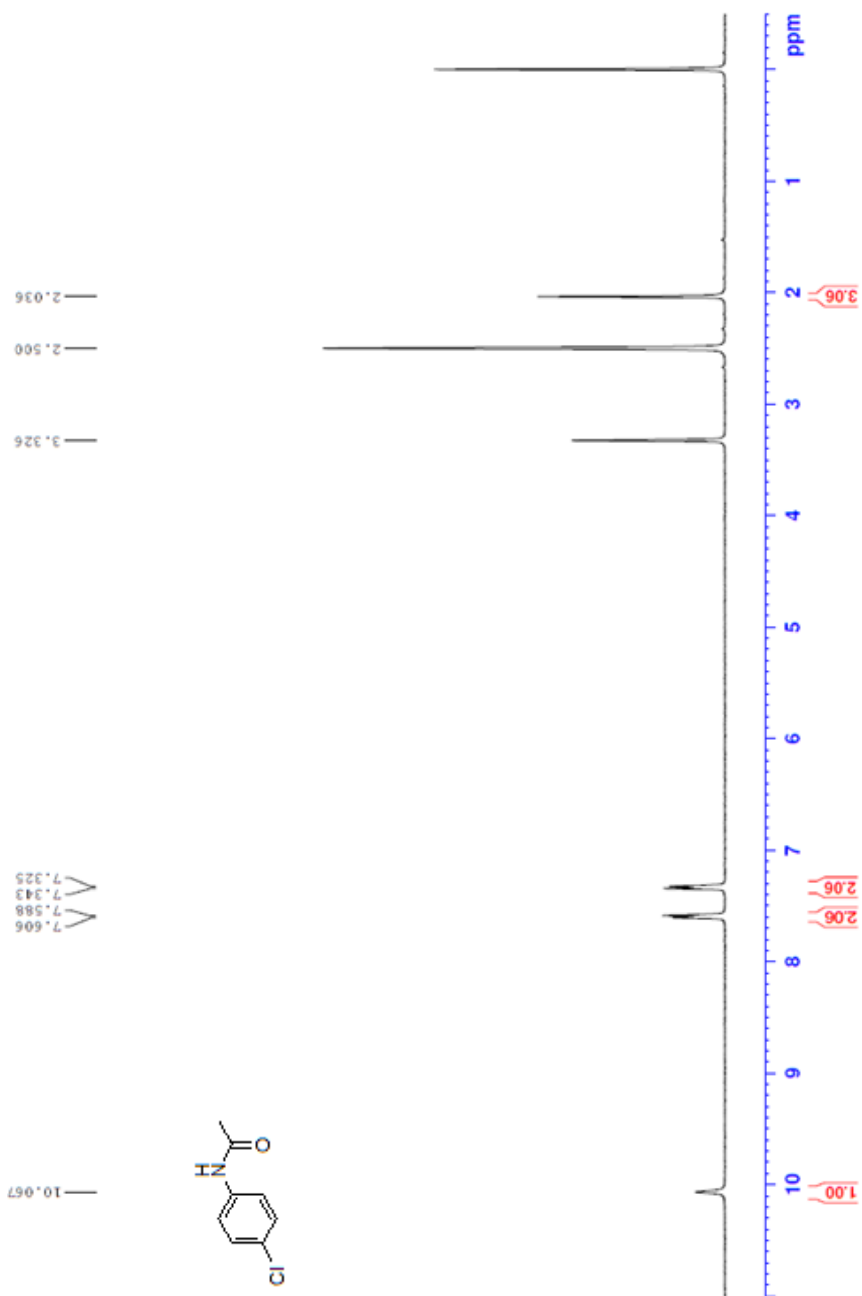


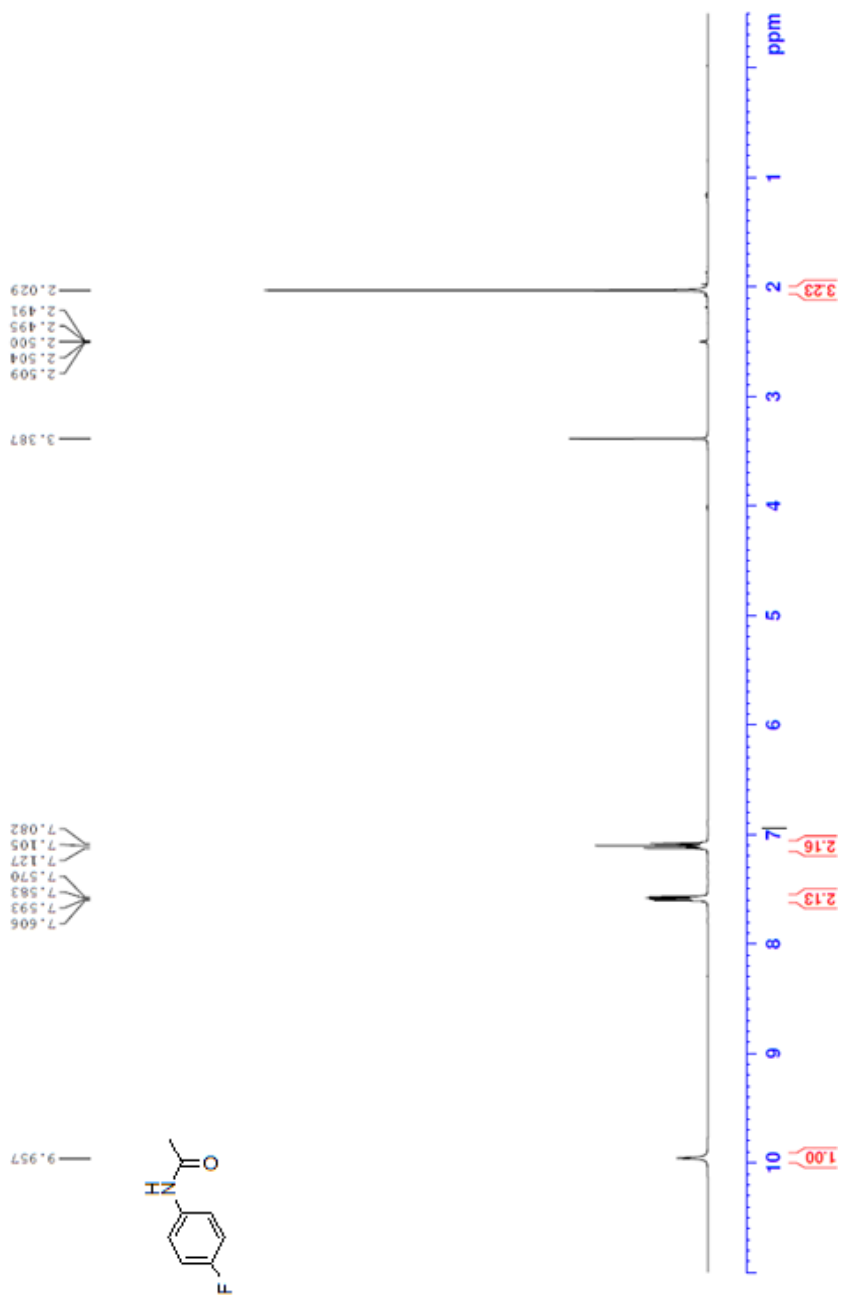


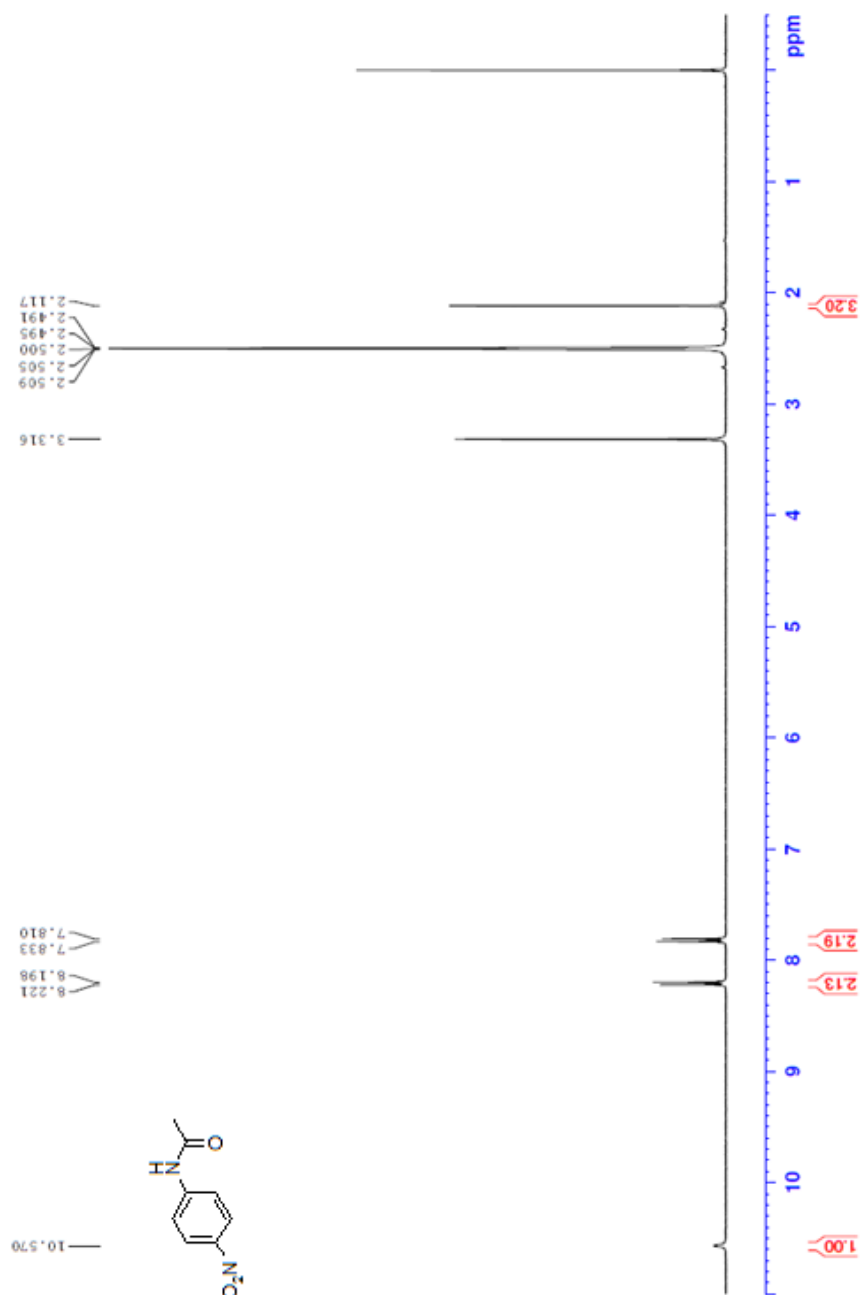


400 MHz ^1H NMR spectrum ($\text{DMSO-}d_6$) of *N*-(4-Iodophenyl)acetamide

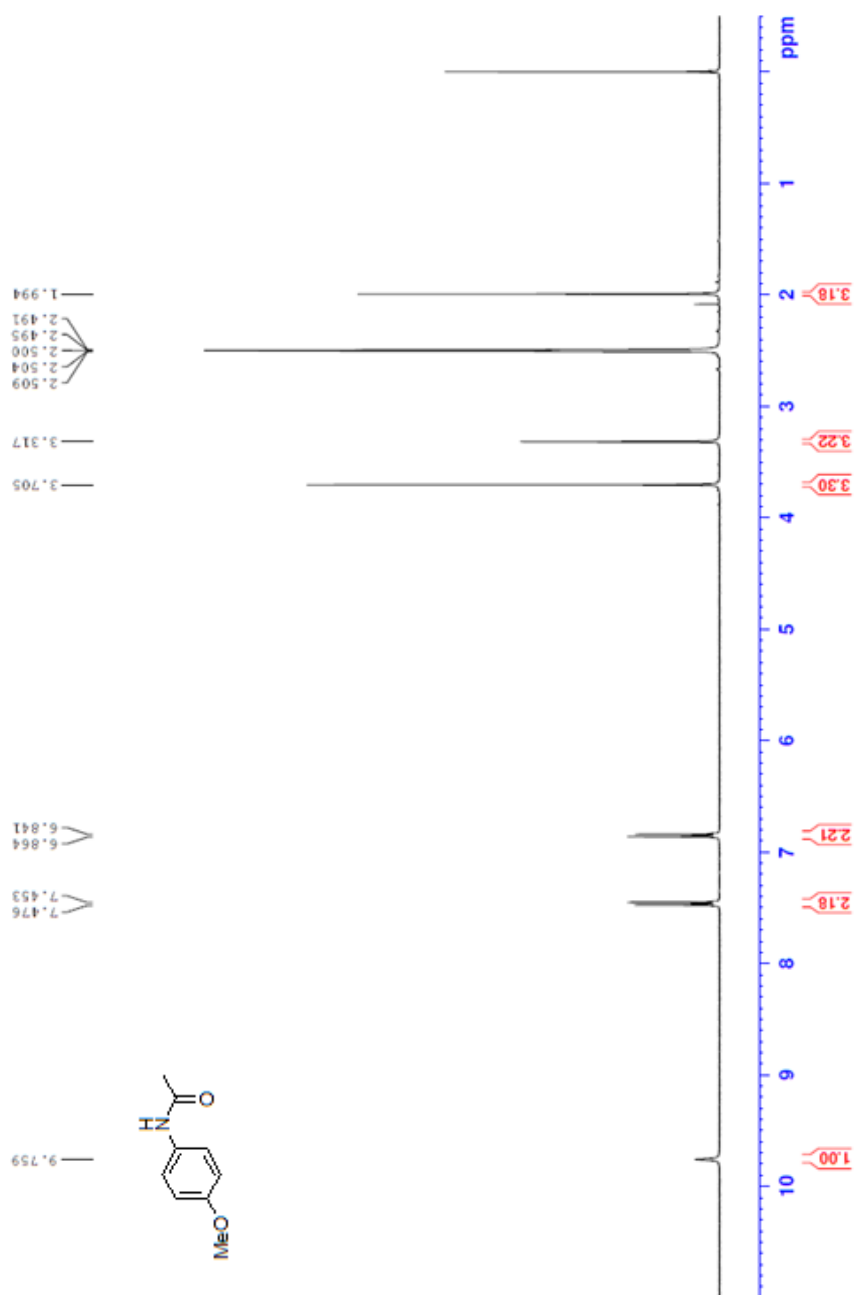


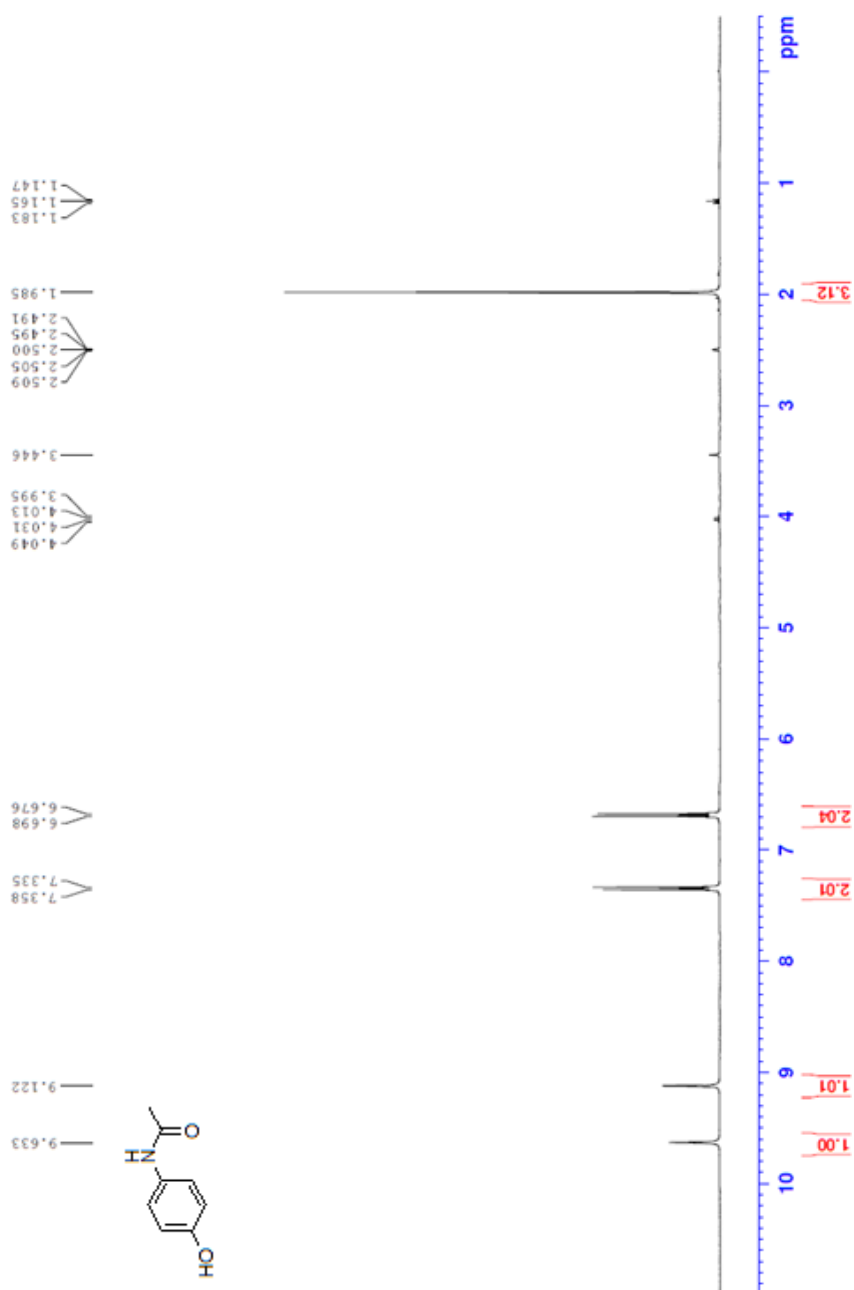






400 MHz ¹H NMR spectrum (DMSO-*d*₆) of *N*-(4-Nitrophenyl)acetamide

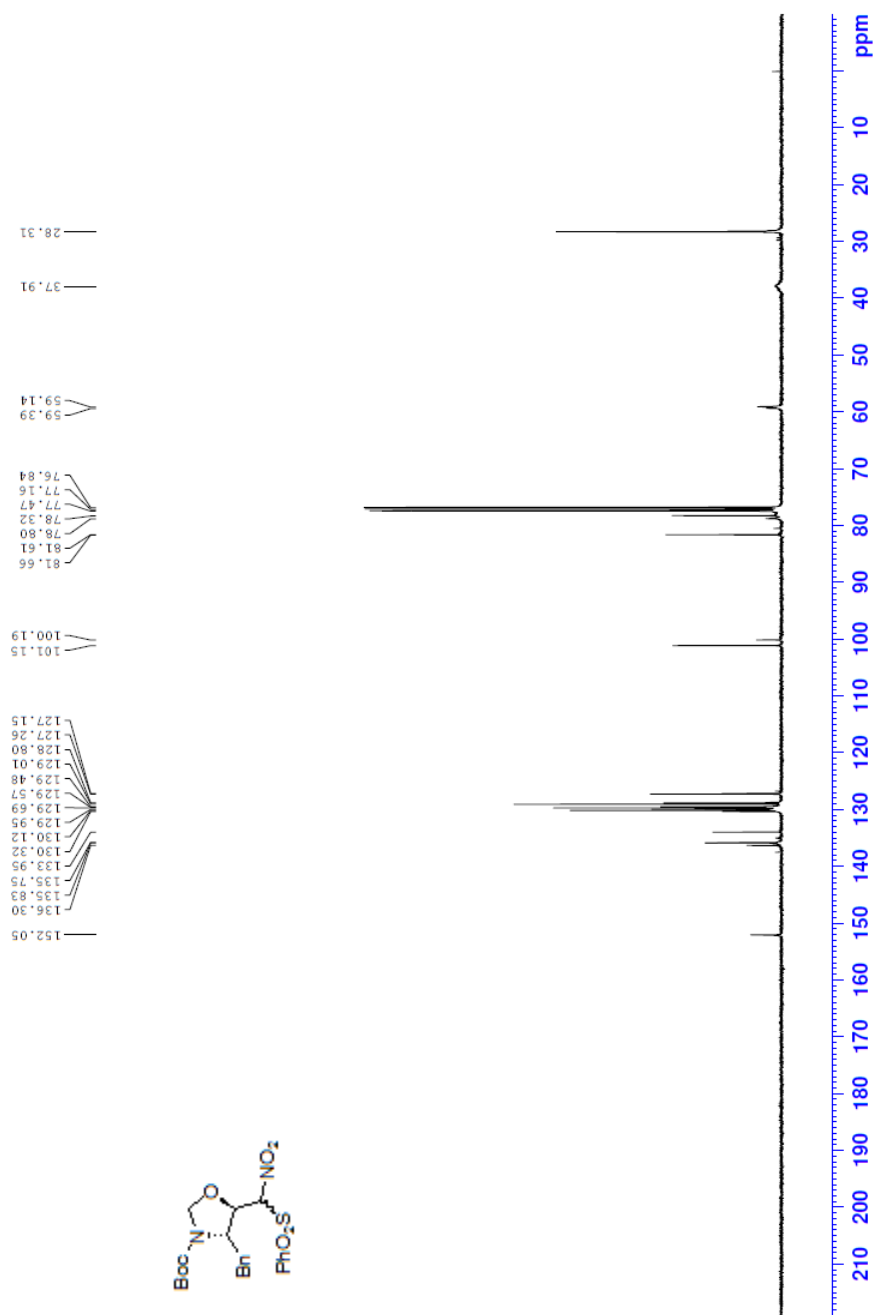
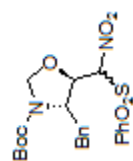


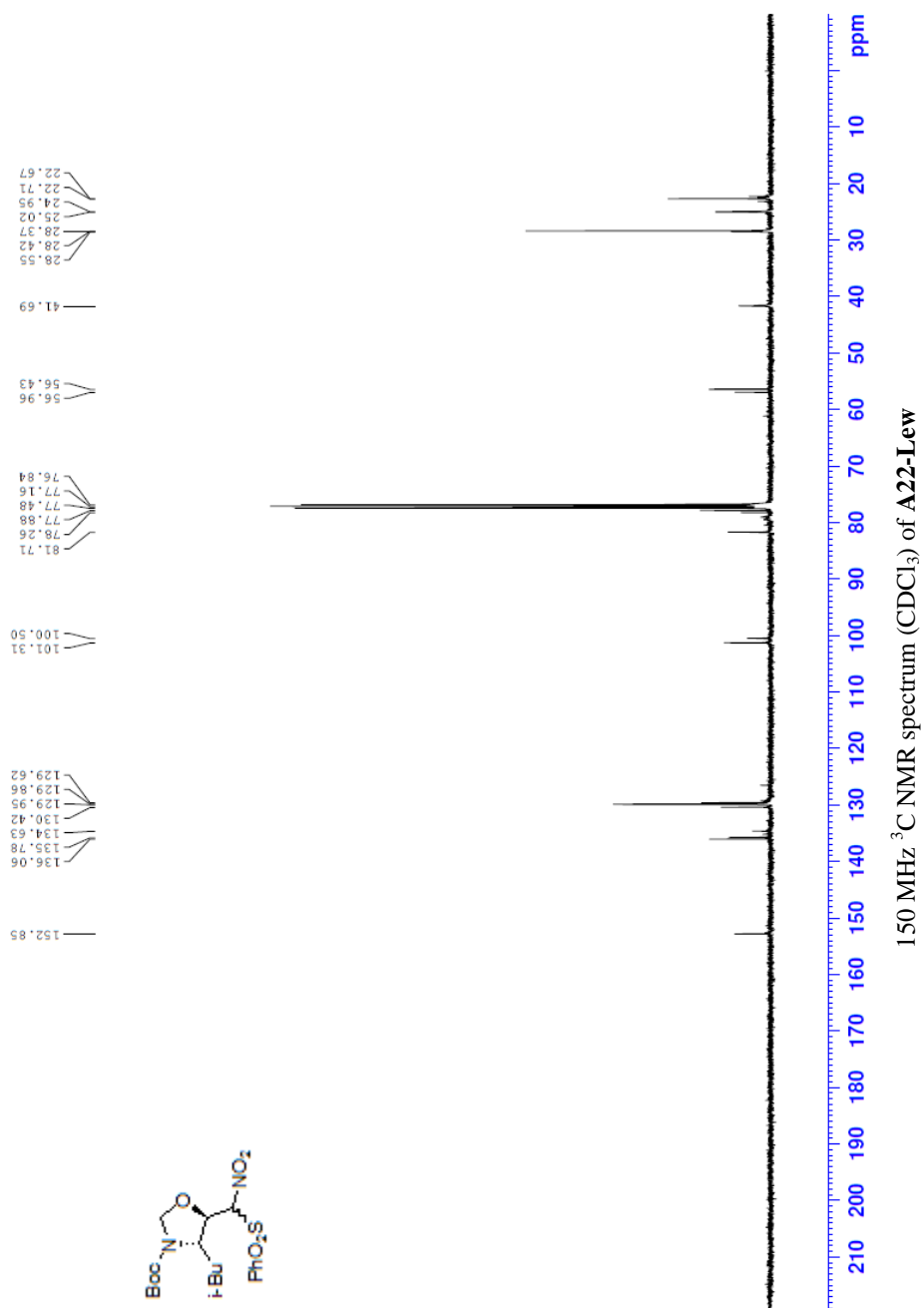


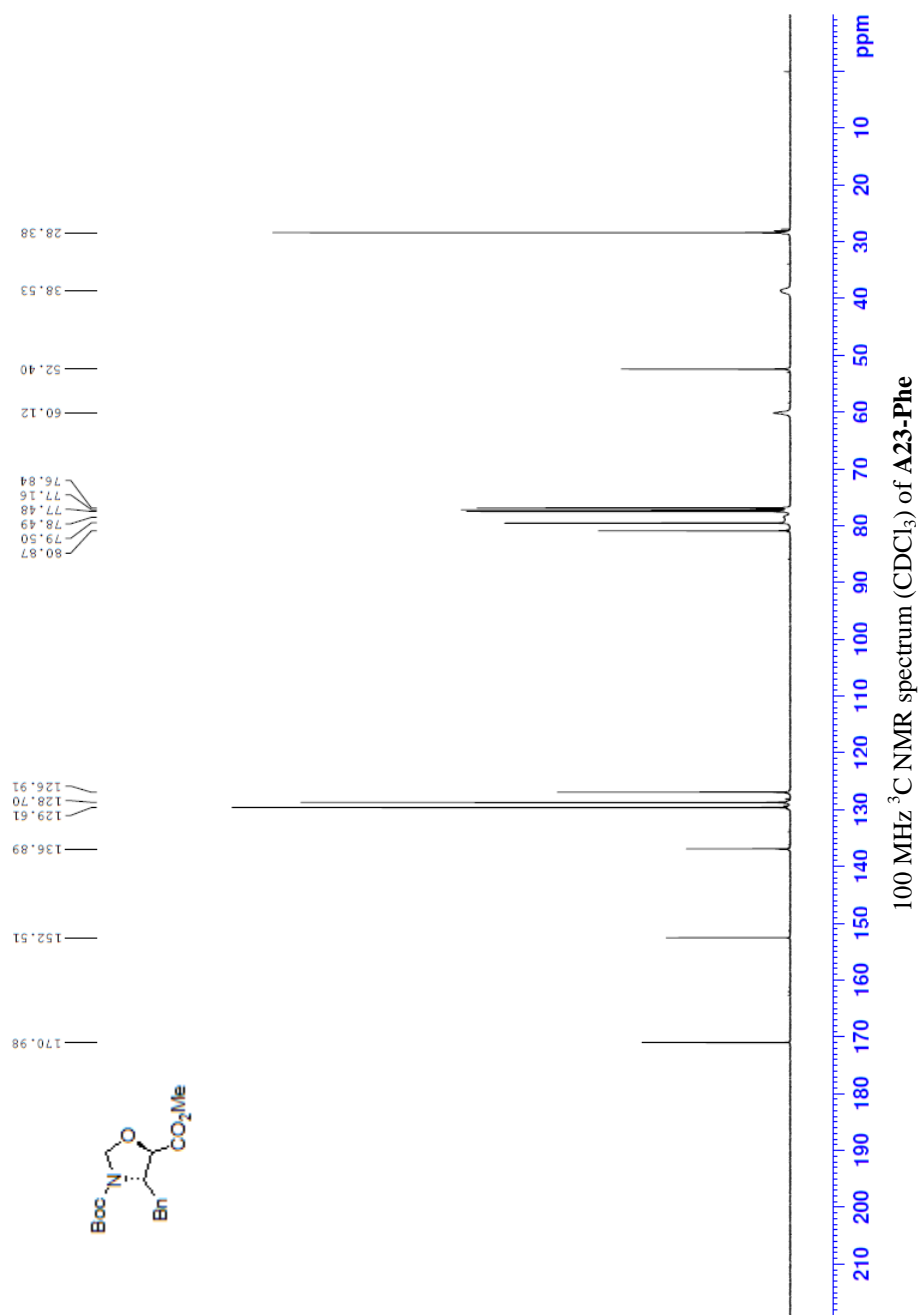
List of ^{13}C -NMR Spectra of Selected Compounds

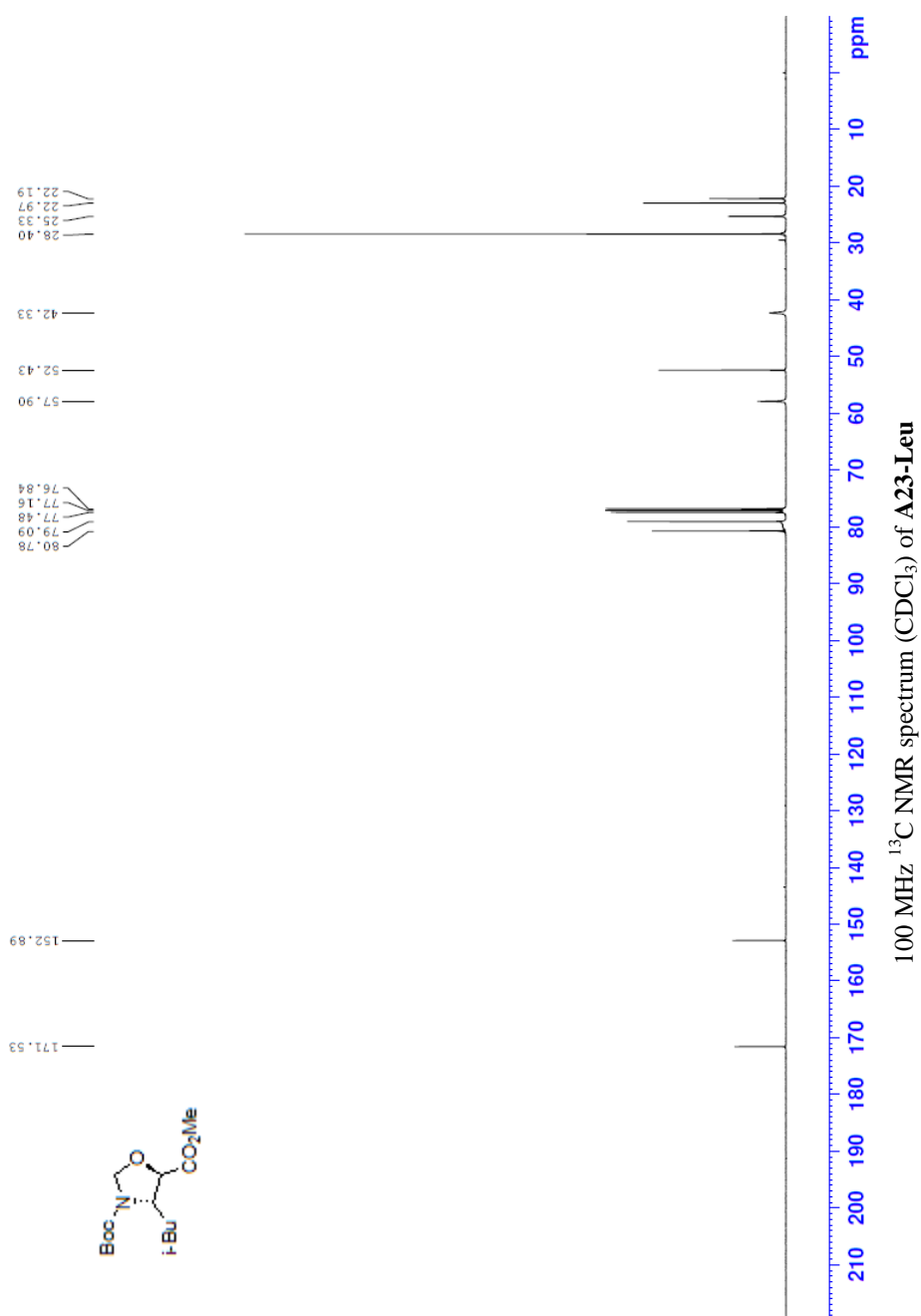
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4. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A23-Leu	139
5. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A23-Val	140
6. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A23-Ala	141
7. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A23-Ser	142
8. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A24-Phe	143
9. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A24-Leu	144
10. 100 MHz ^{13}C -NMR spectrum ($\text{DMSO}-d_6$) of (2 <i>S</i> ,3 <i>R</i>)-AHPBA ...	145
11. 100 MHz ^{13}C -NMR spectrum ($\text{MeOH}-d_4$) of (2 <i>S</i> ,3 <i>R</i>)-AHMHA ..	146
12. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of <i>ent</i> - A25-Phe	147
13. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A28-Phe	148
14. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A28-Phe'-Val	149
15. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A28-Leu	150
16. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A28-Val	151
17. 100 MHz ^{13}C -NMR spectrum ($\text{MeOH}-d_4$) of A29	152
18. 100 MHz ^{13}C -NMR spectrum ($\text{MeOH}-d_4$) of A30	153
19. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A31	154
20. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A32	155
21. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of A33	156
22. 100 MHz ^{13}C -NMR spectrum (DCl) of L-TBOA	157
23. 100 MHz ^{13}C -NMR spectrum (CDCl_3) of <i>ent</i> - A34	158

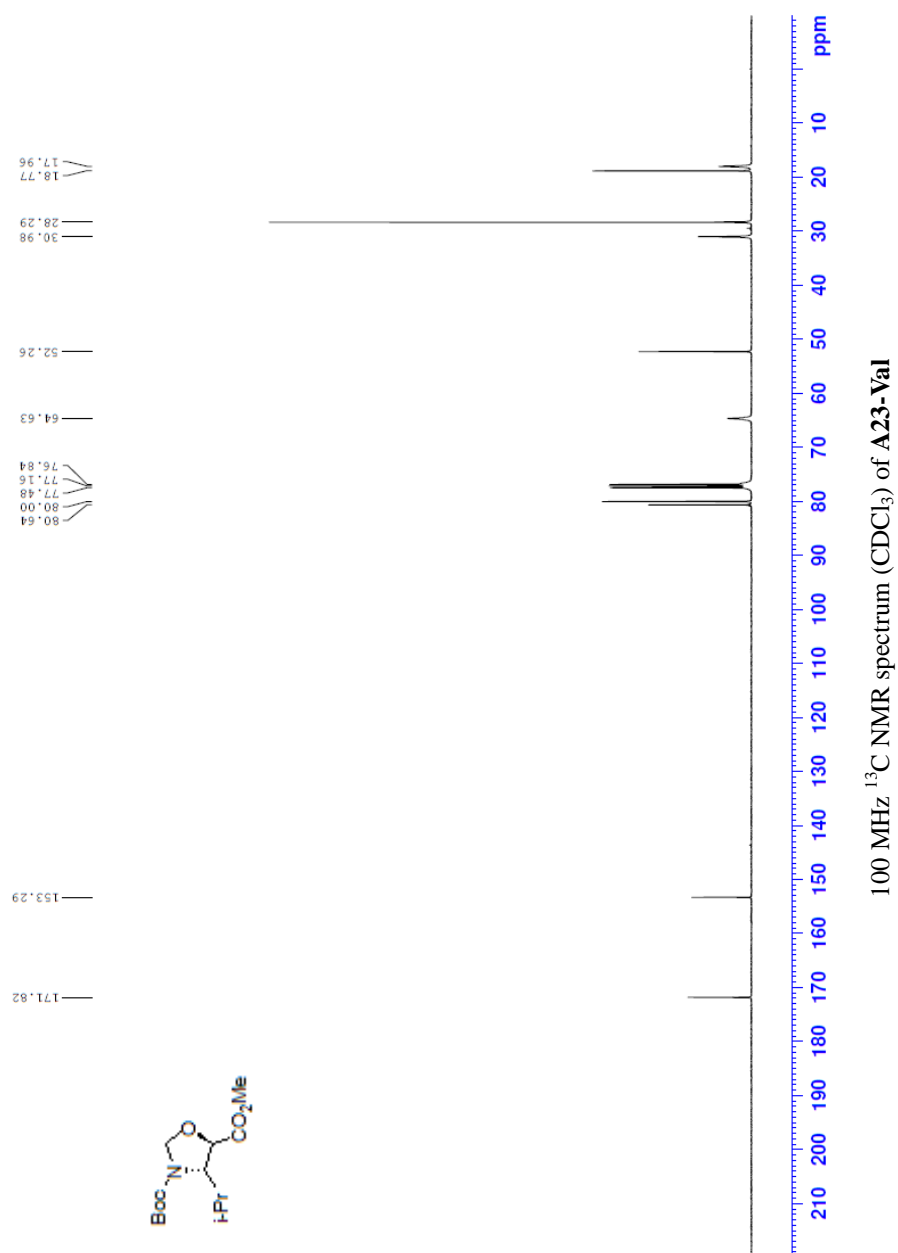
24. 100 MHz ^{13}C -NMR spectrum (DMSO- d_6) of A35	159
25. 100 MHz ^{13}C -NMR spectrum (DMSO- d_6) of <i>N</i> -(4-Iodophenyl)- acetamide	160
26. 100 MHz ^{13}C -NMR spectrum (DMSO- d_6) of <i>N</i> -(4-Bromophenyl)- acetamide	161
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28. 100 MHz ^{13}C -NMR spectrum (DMSO- d_6) of <i>N</i> -(4-Fluorophenyl)- acetamide	163
29. 100 MHz ^{13}C -NMR spectrum (DMSO- d_6) of <i>N</i> -(4-Nitrophenyl)- acetamide	164
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31. 100 MHz ^{13}C -NMR spectrum (DMSO- d_6) of <i>N</i> -(4- Hydroxyphenyl)-acetamide	165

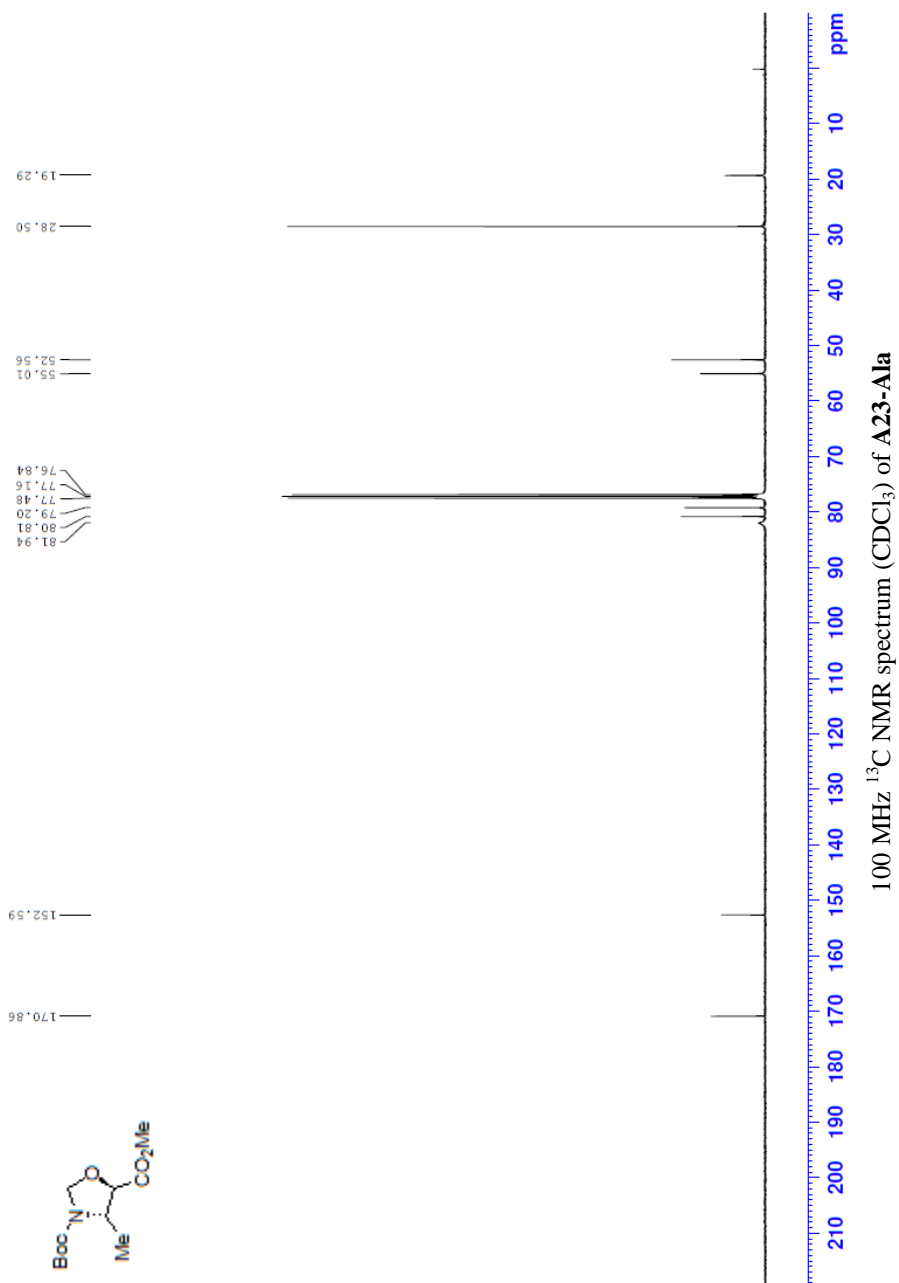


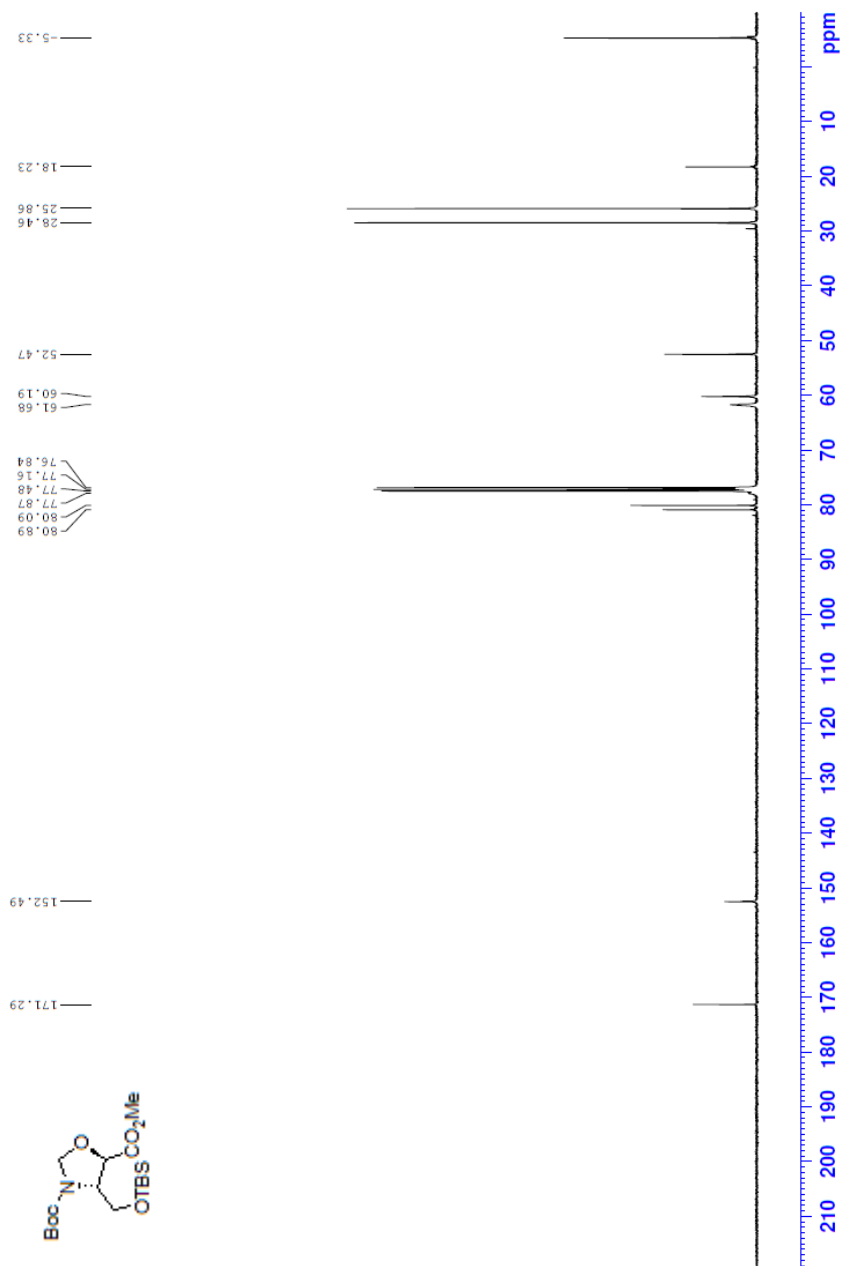


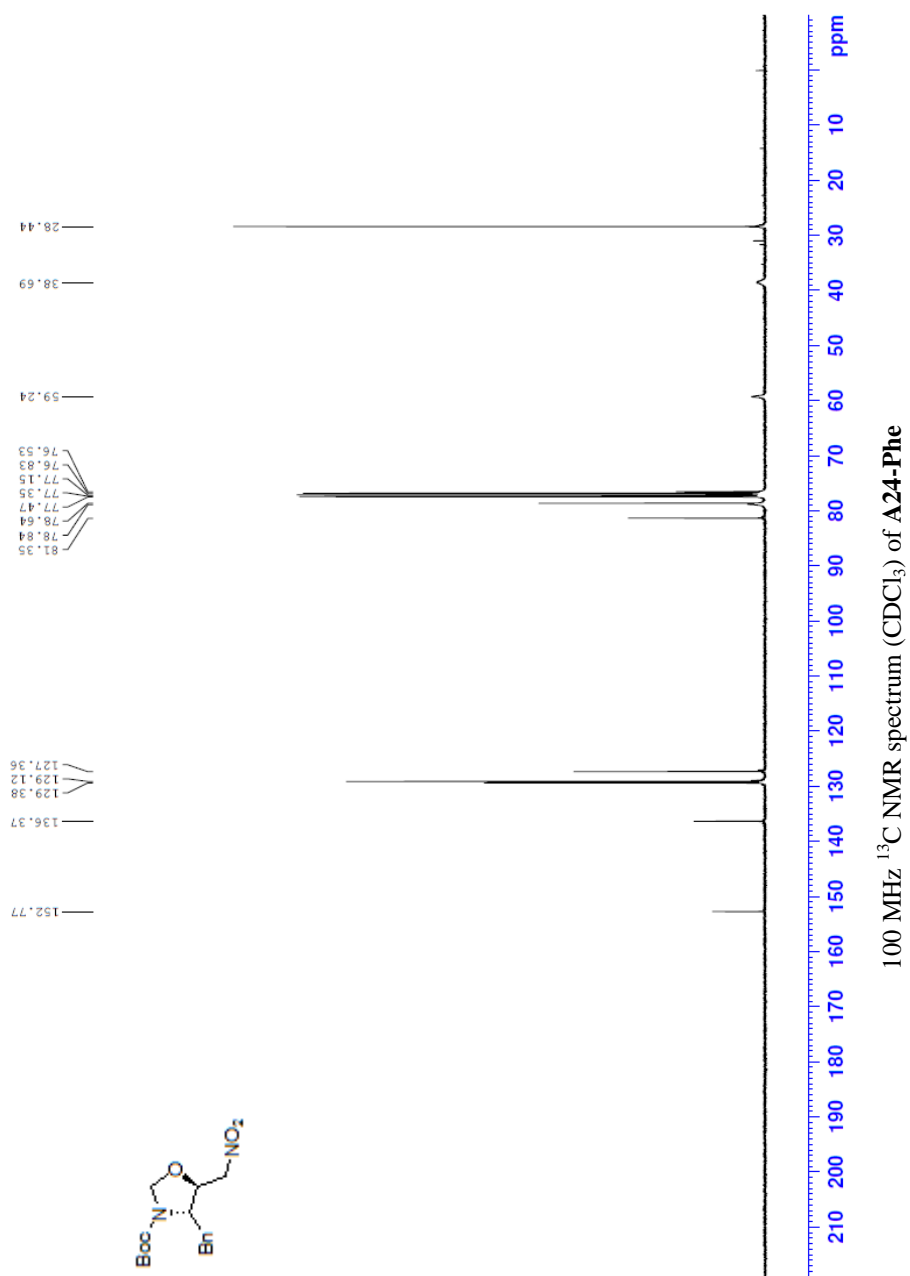


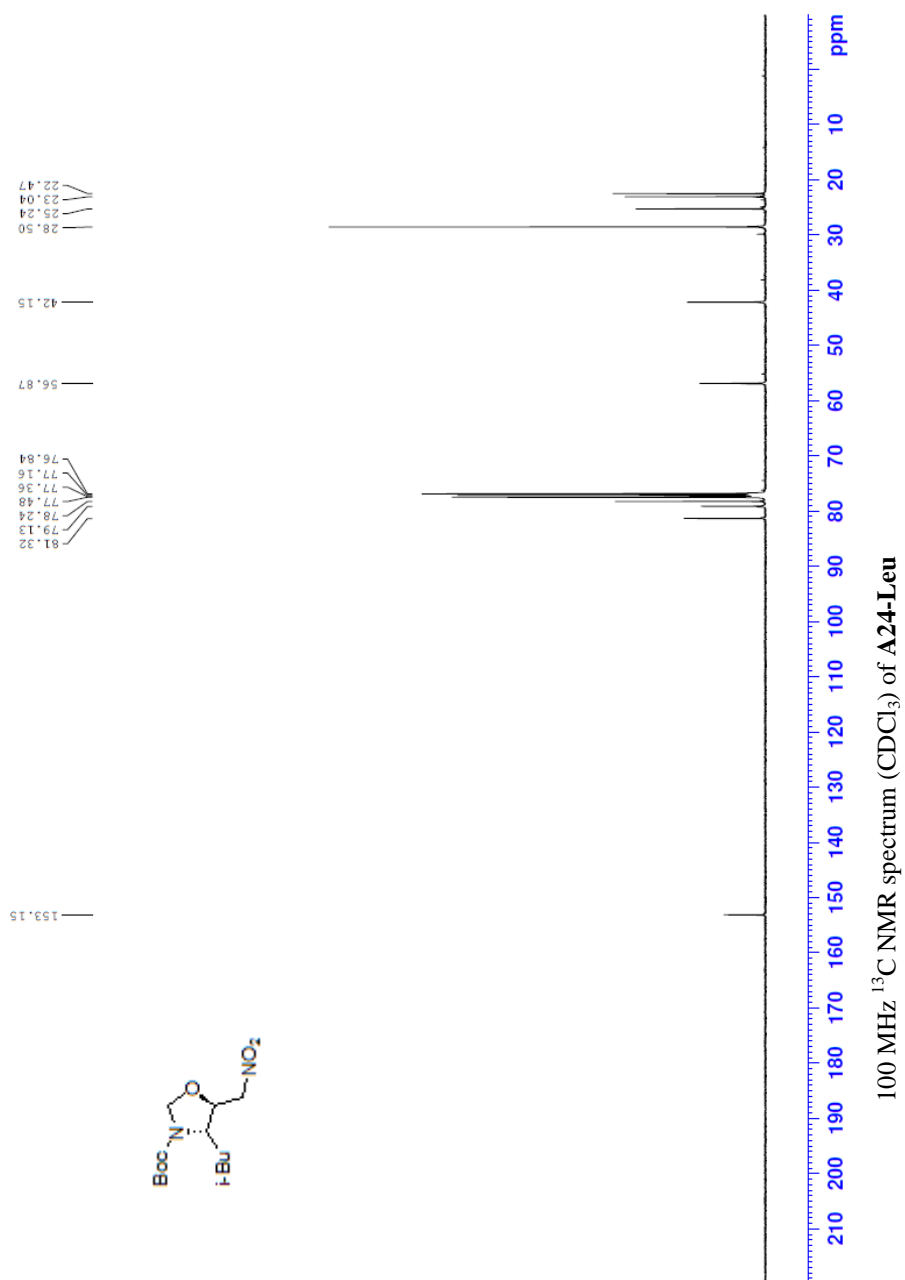


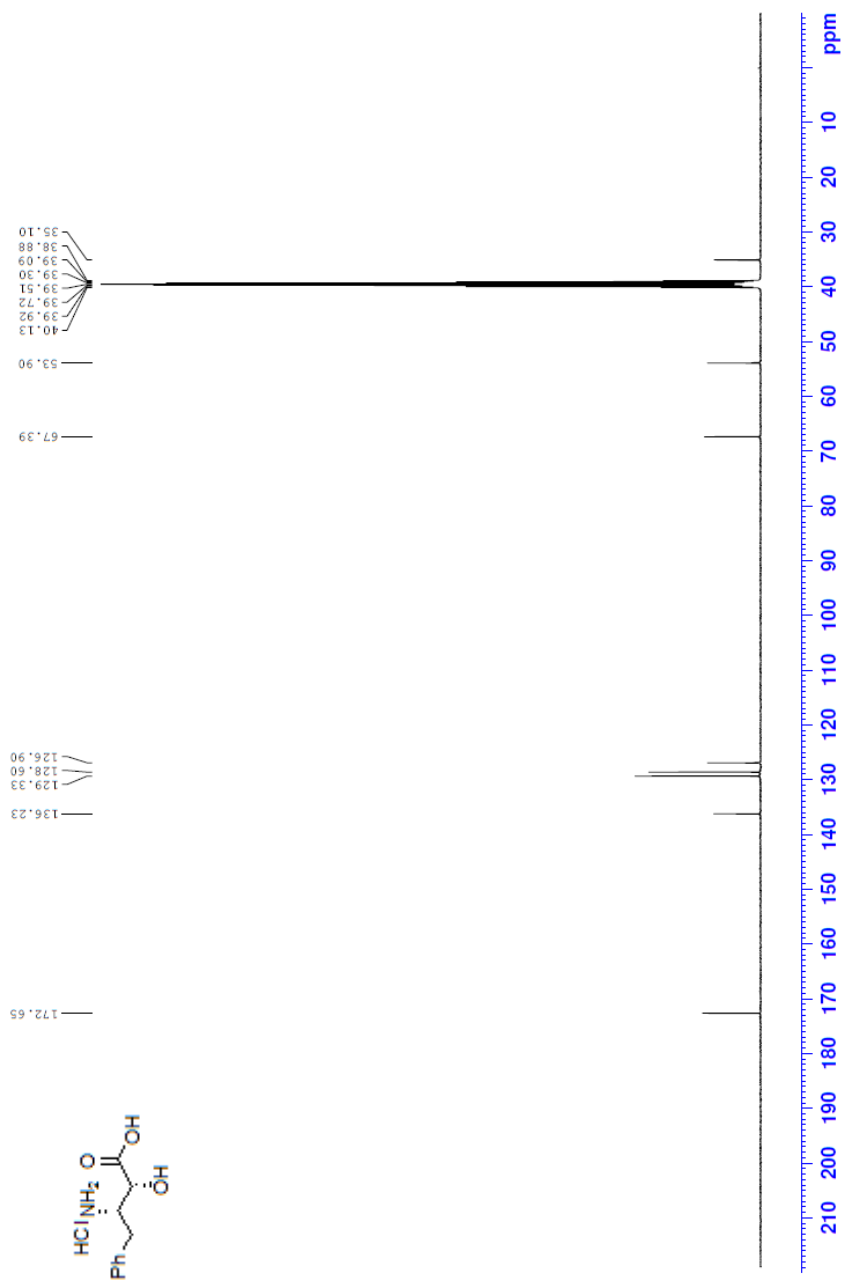


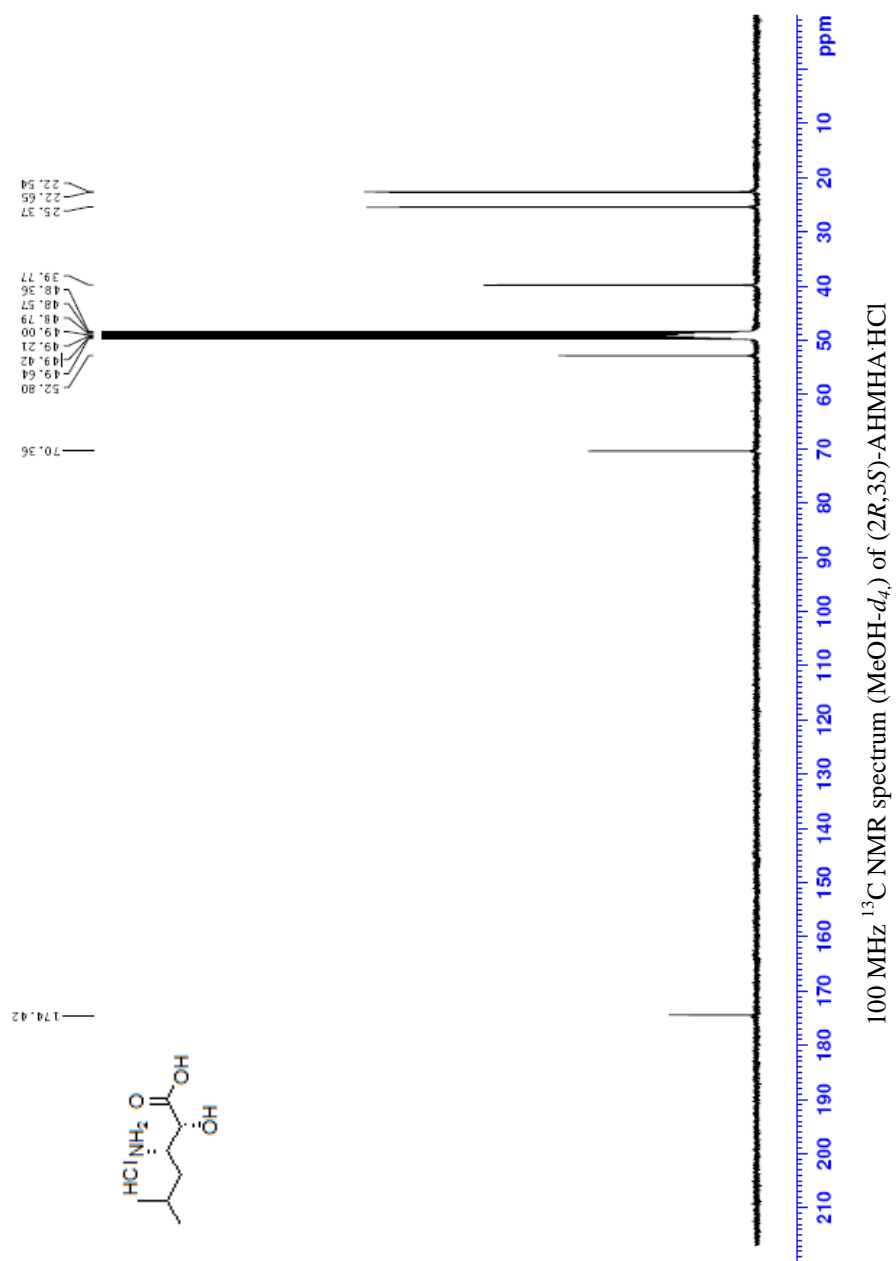


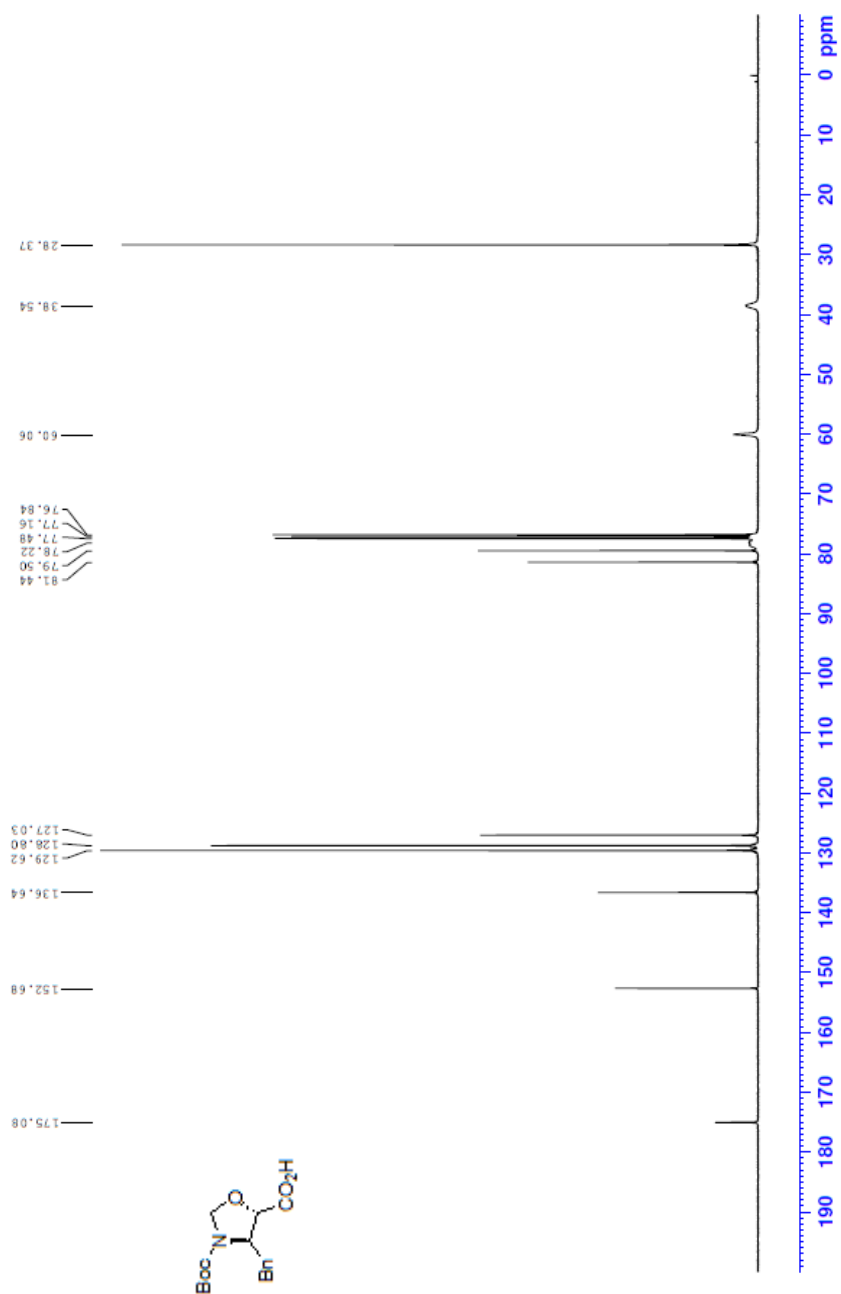
100 MHz ^{13}C NMR spectrum (CDCl_3) of A23-Ser

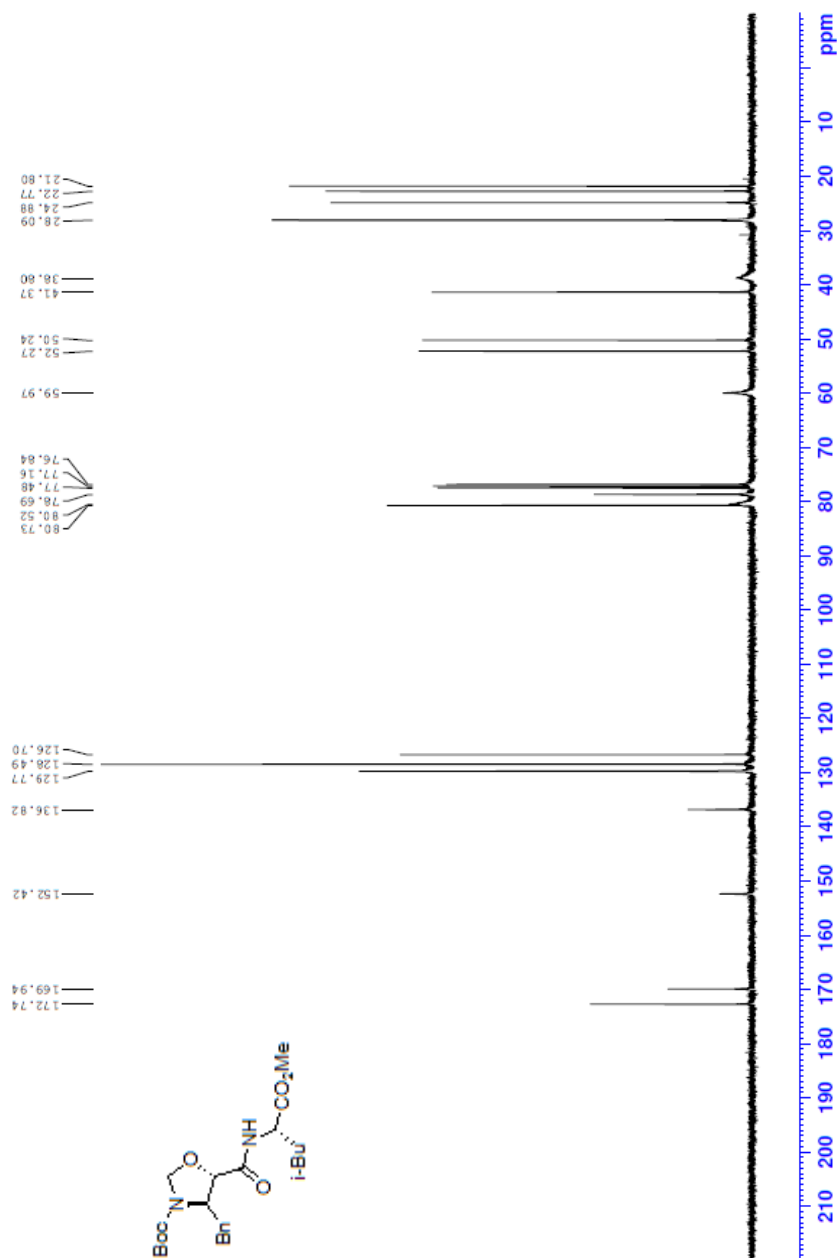




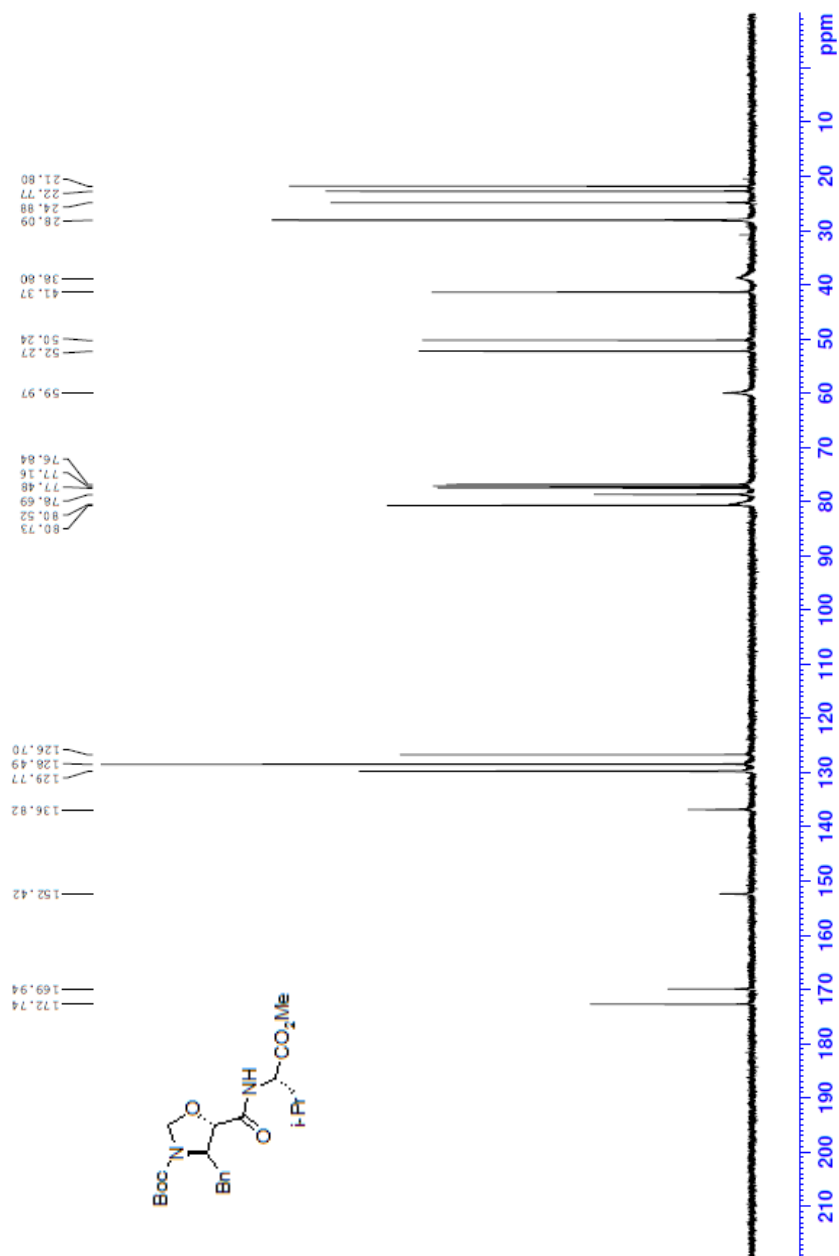




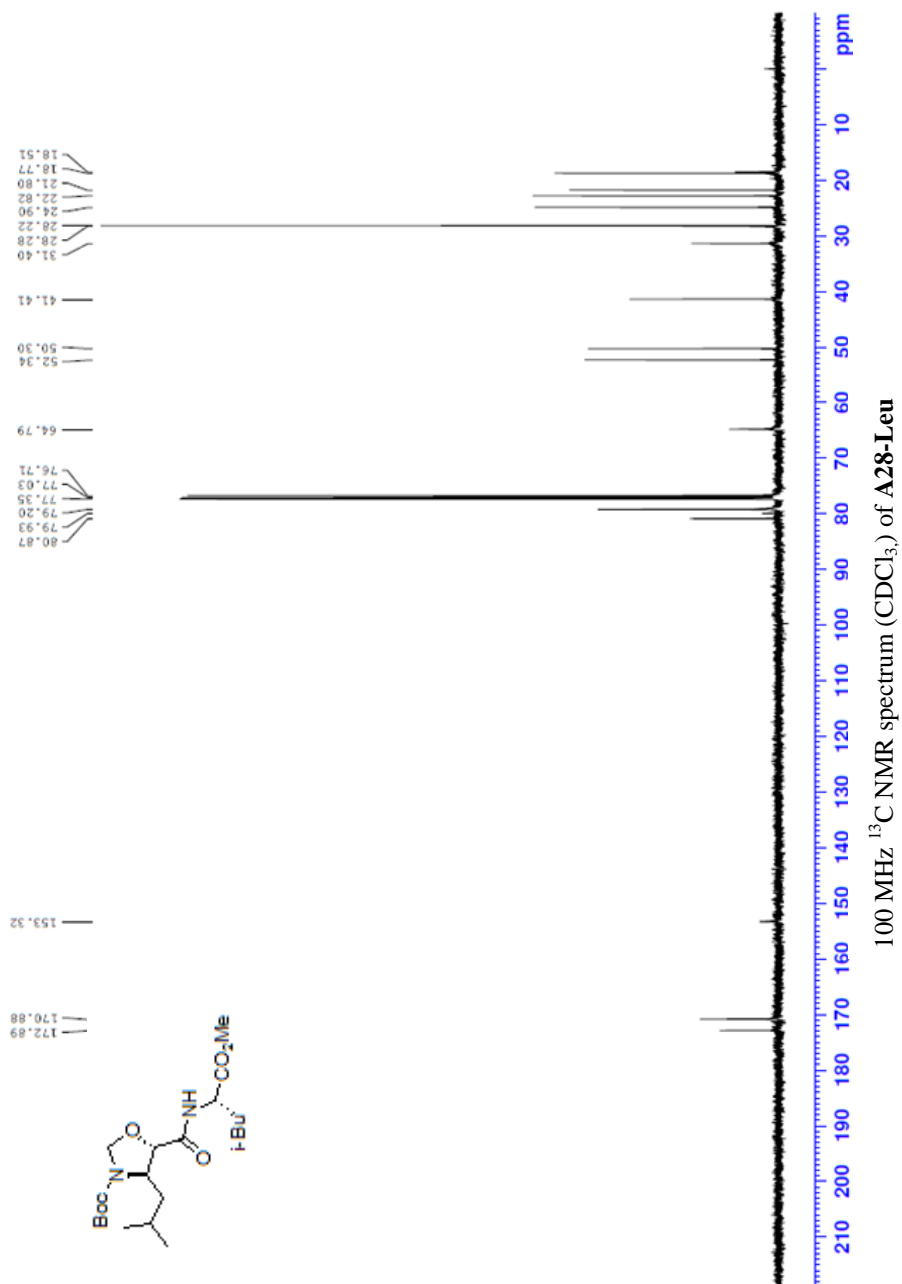


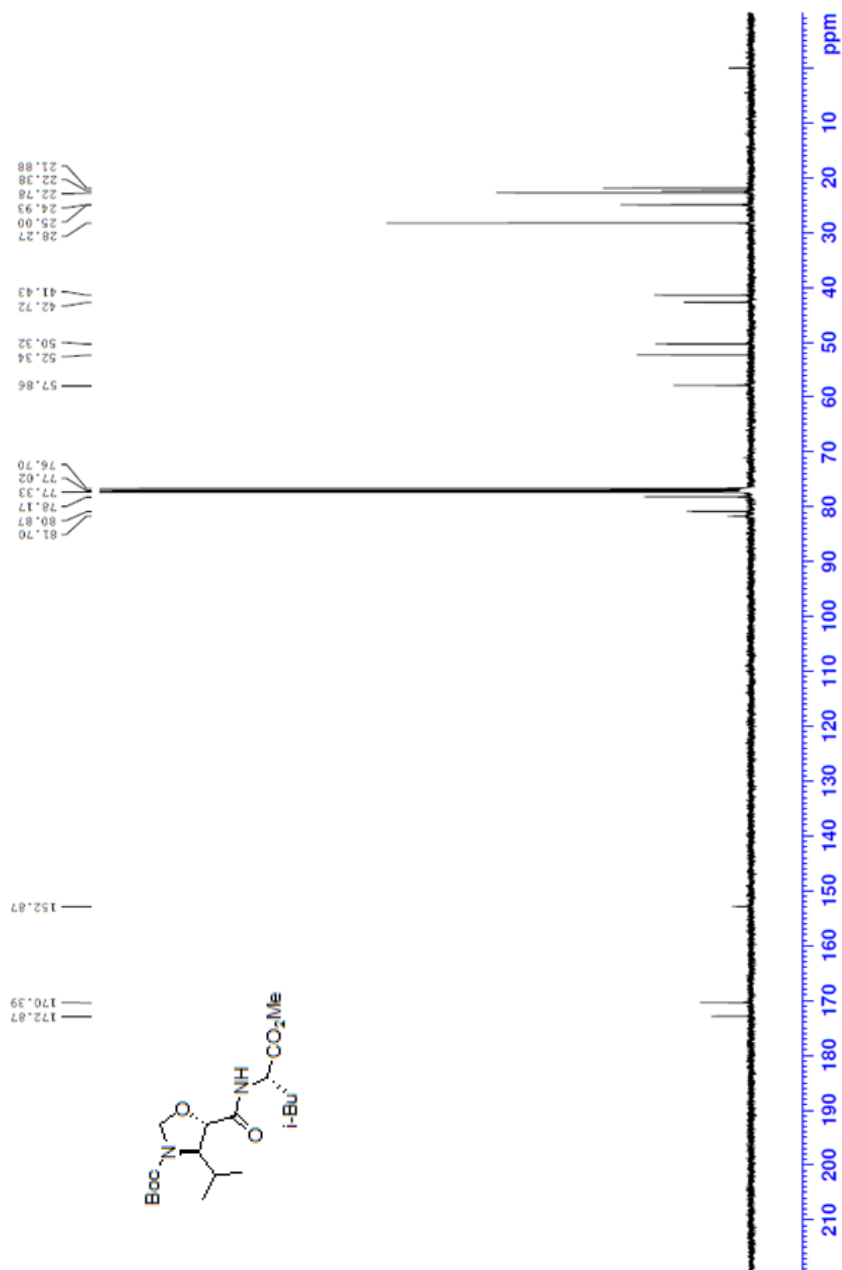


100 MHz ^{13}C NMR spectrum (CDCl_3) of A28-Phe

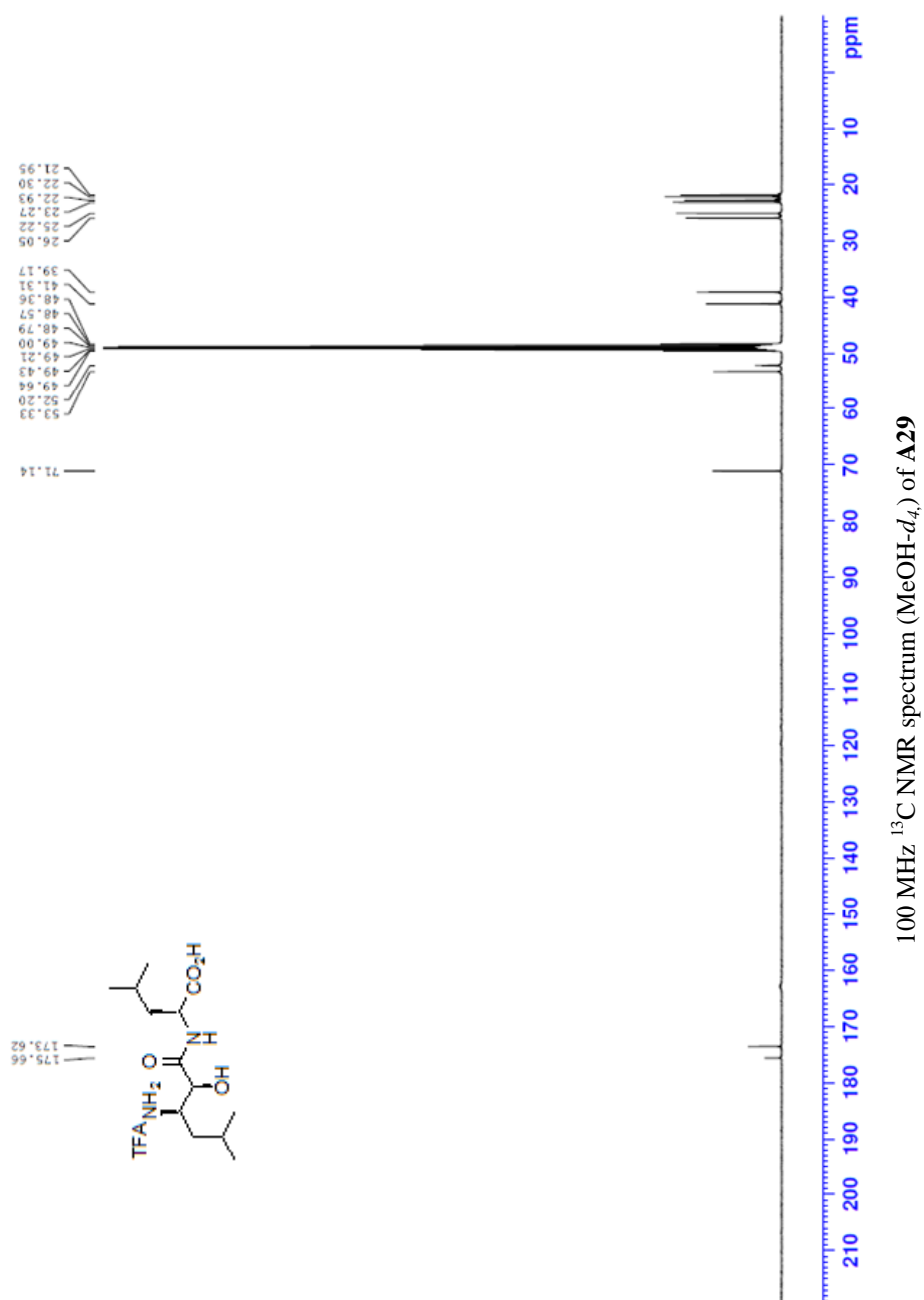


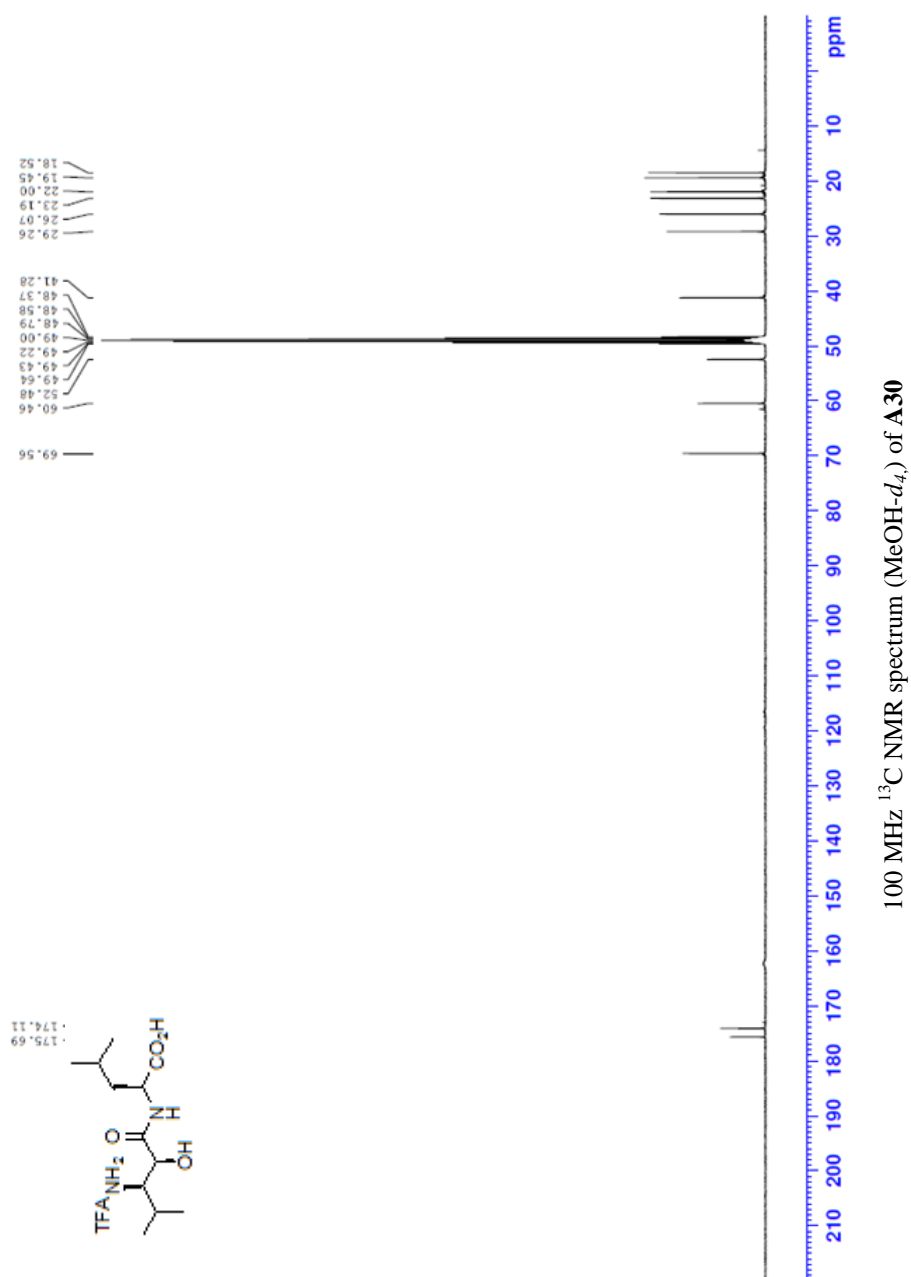
100 MHz ^{13}C NMR spectrum (CDCl_3) of A28-Phe-Val

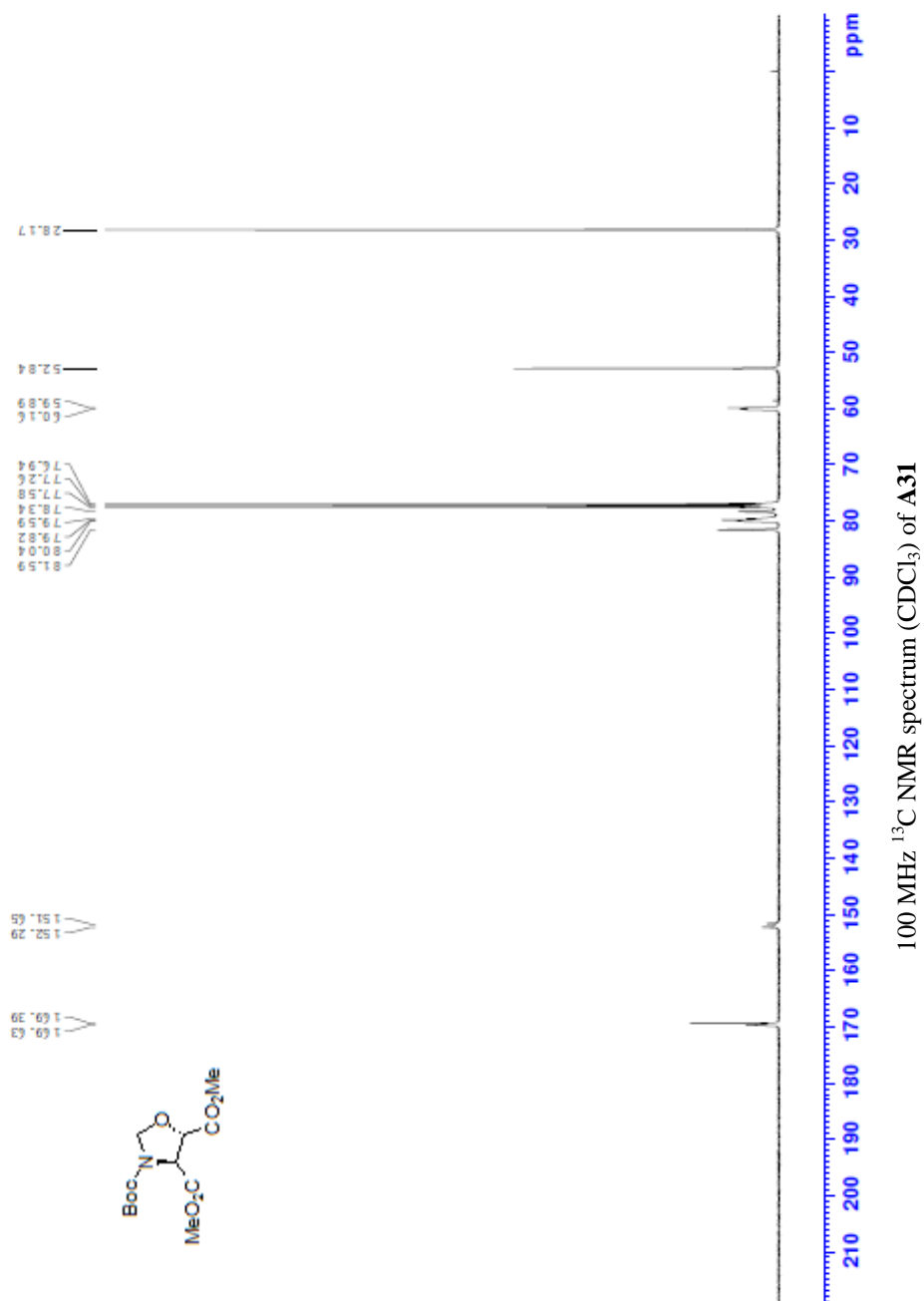


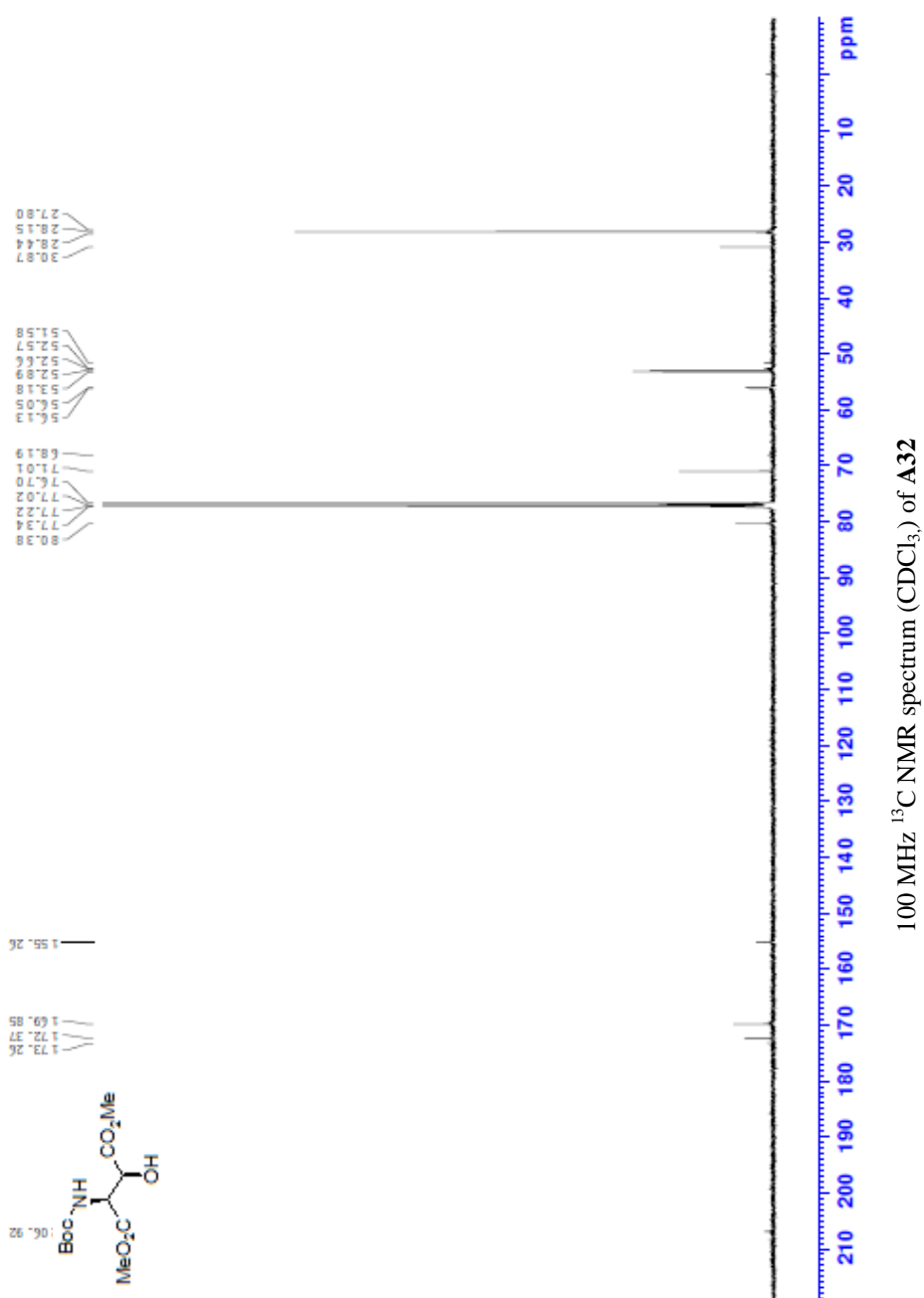


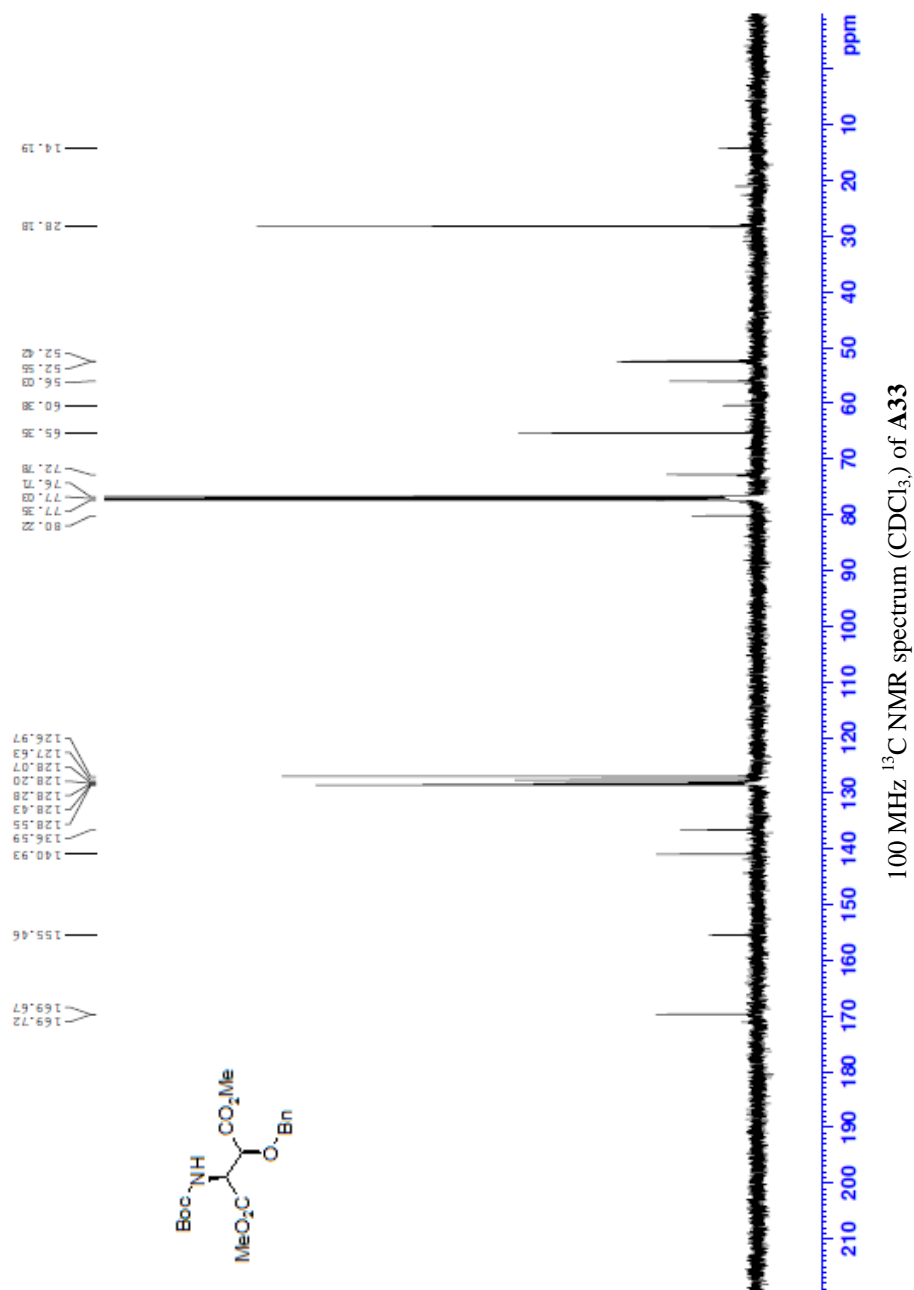
100 MHz ¹³C NMR spectrum (CDCl₃) of A28-Val

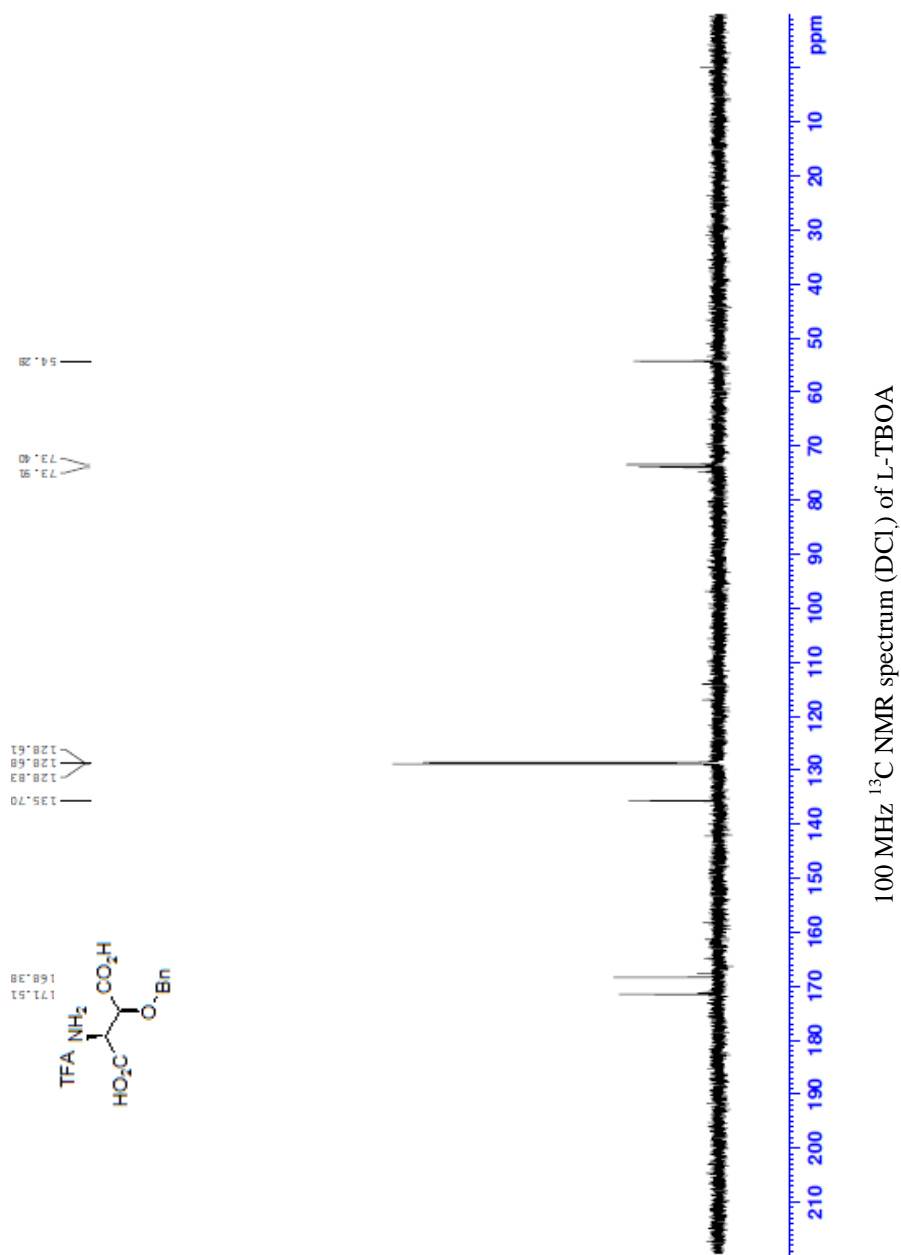


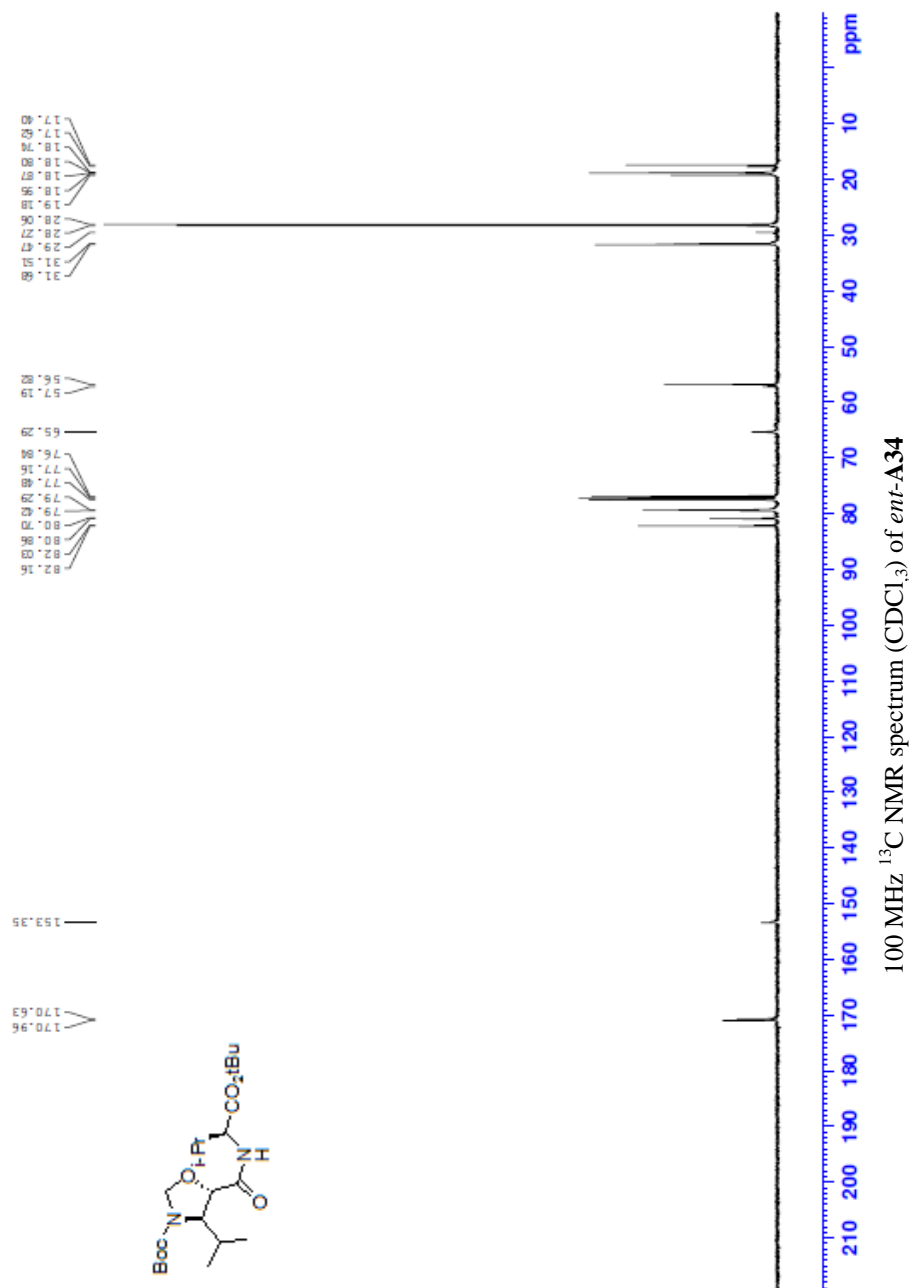


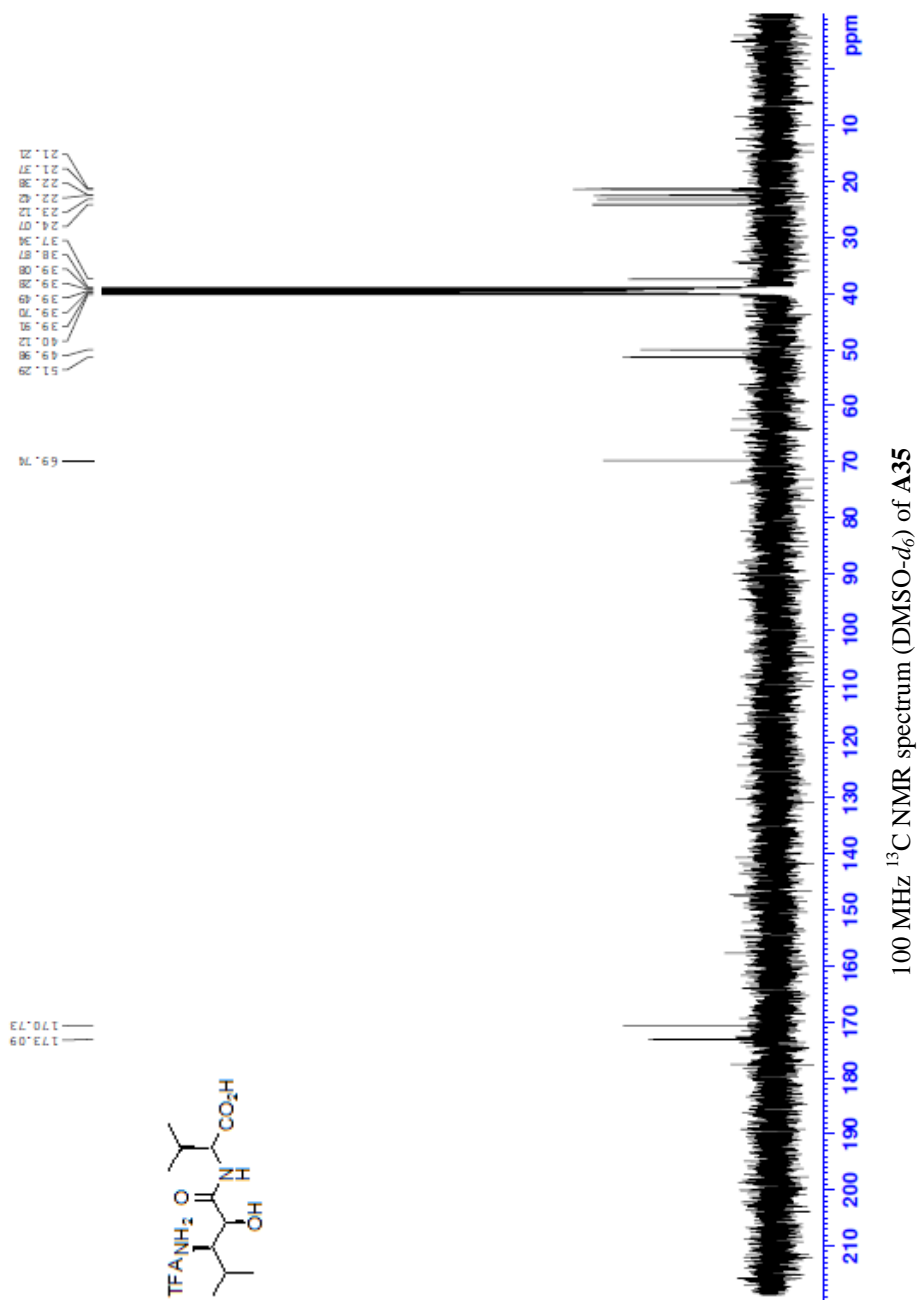


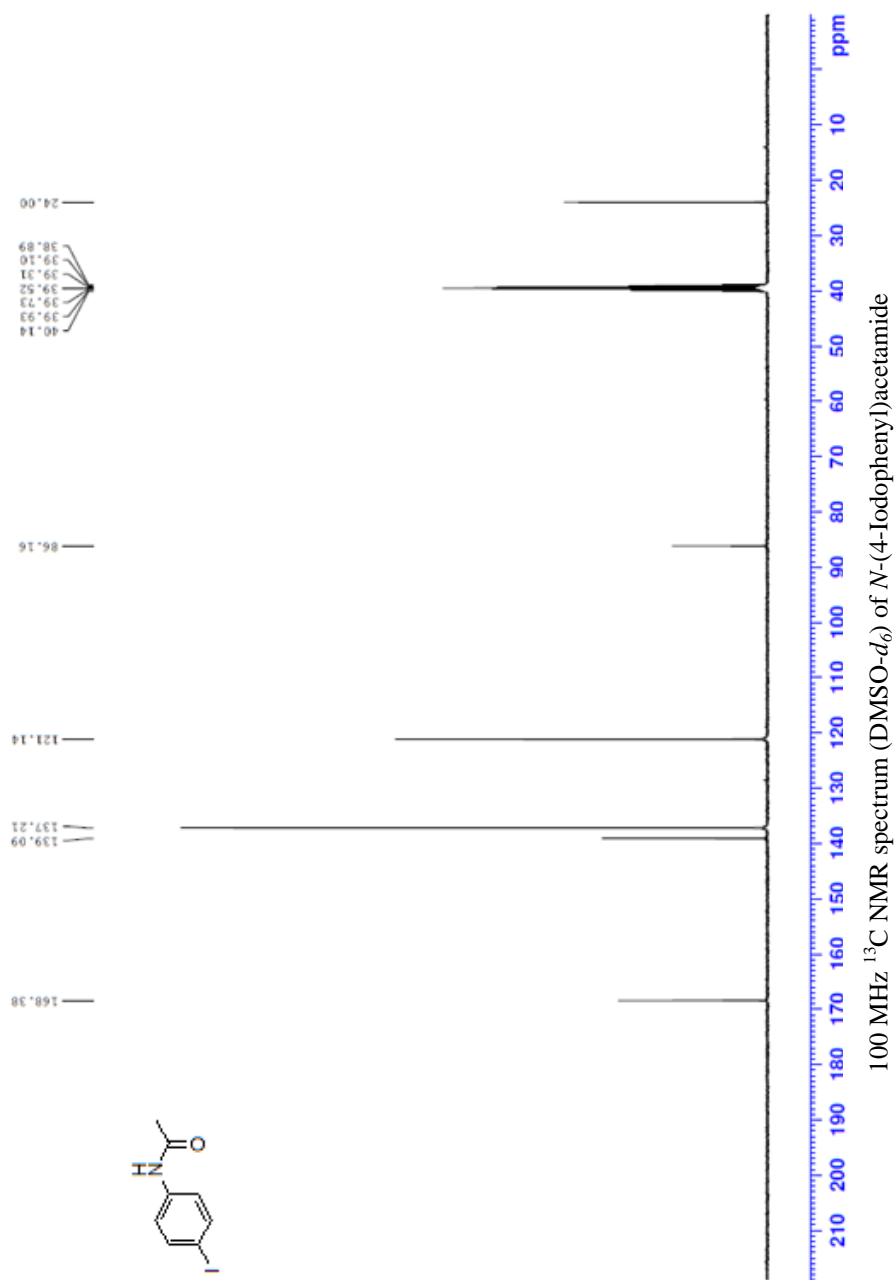


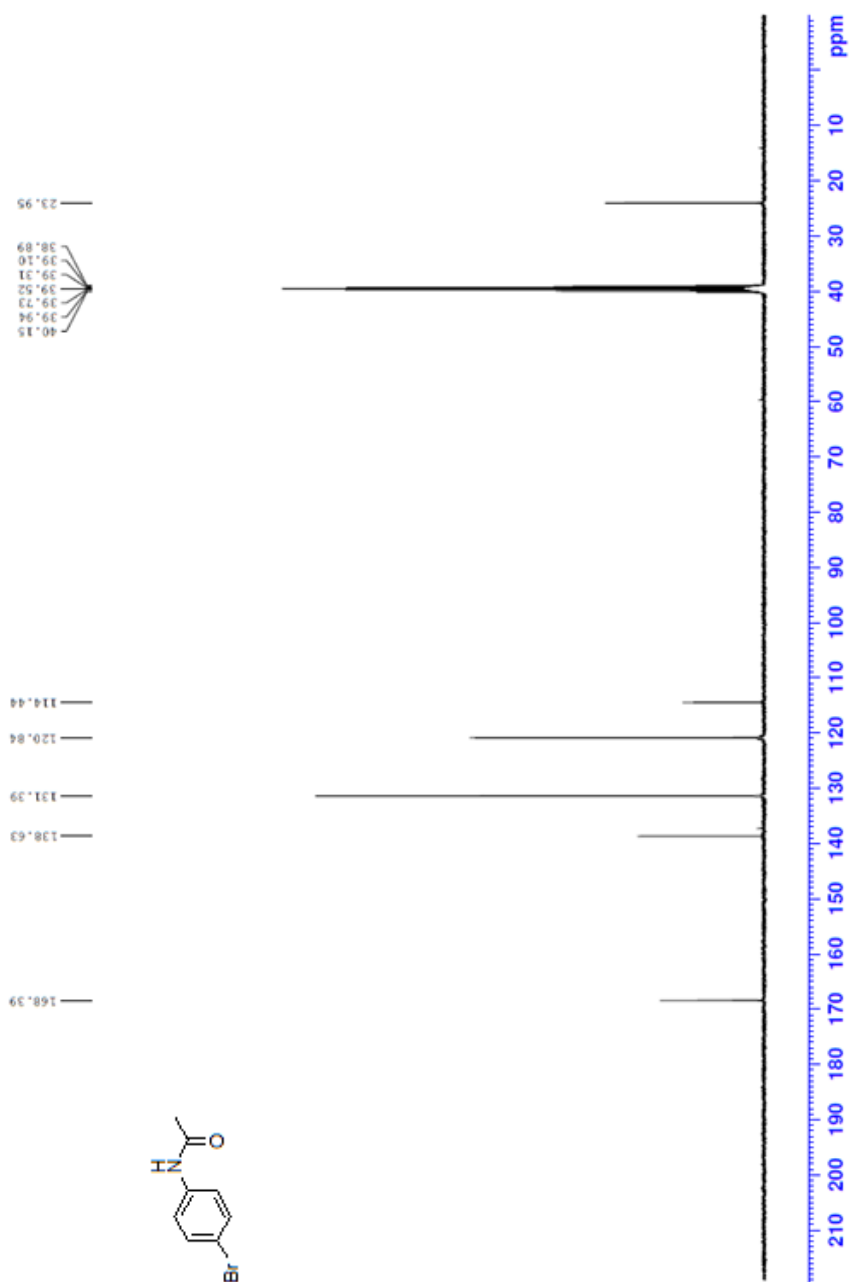


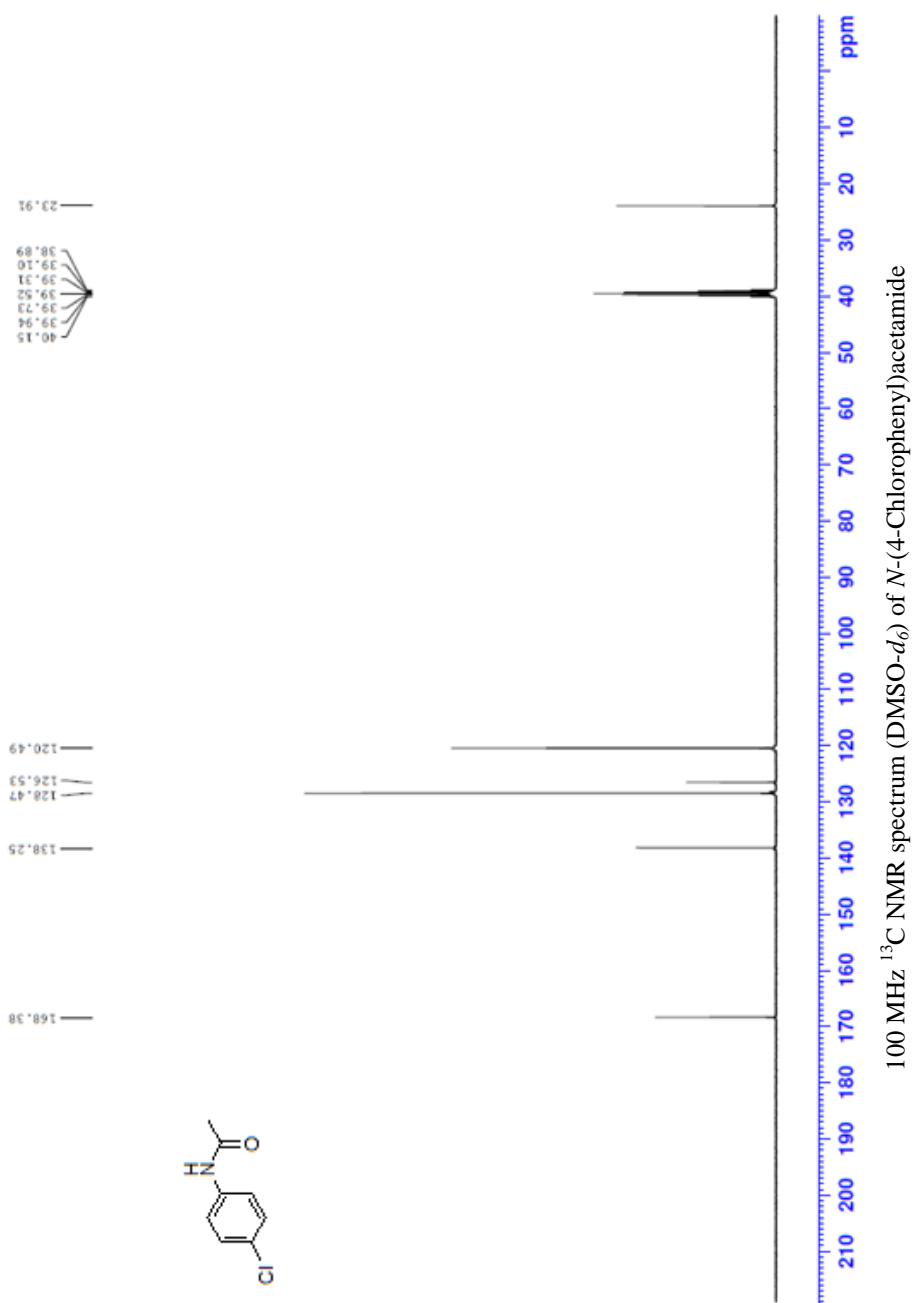


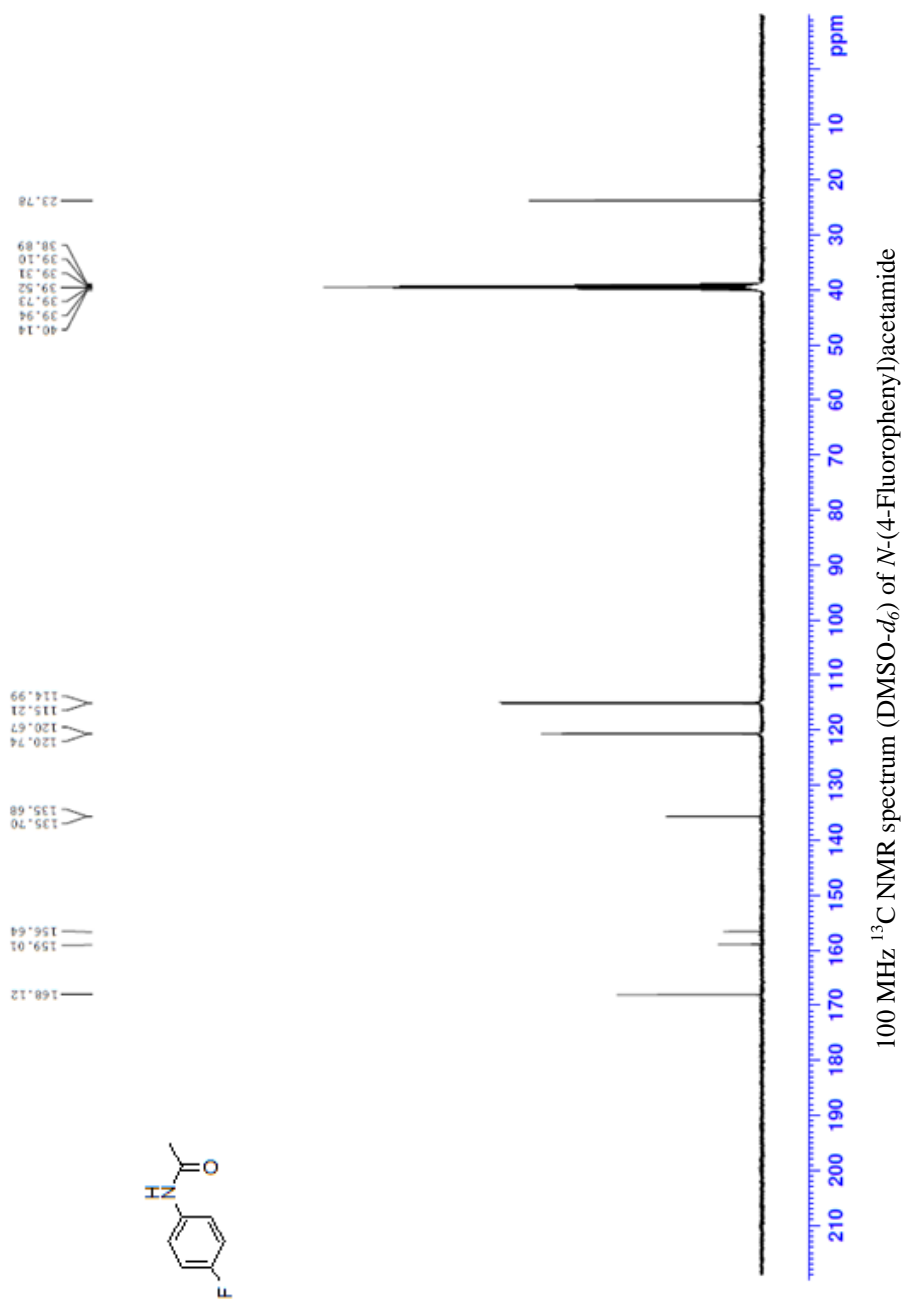


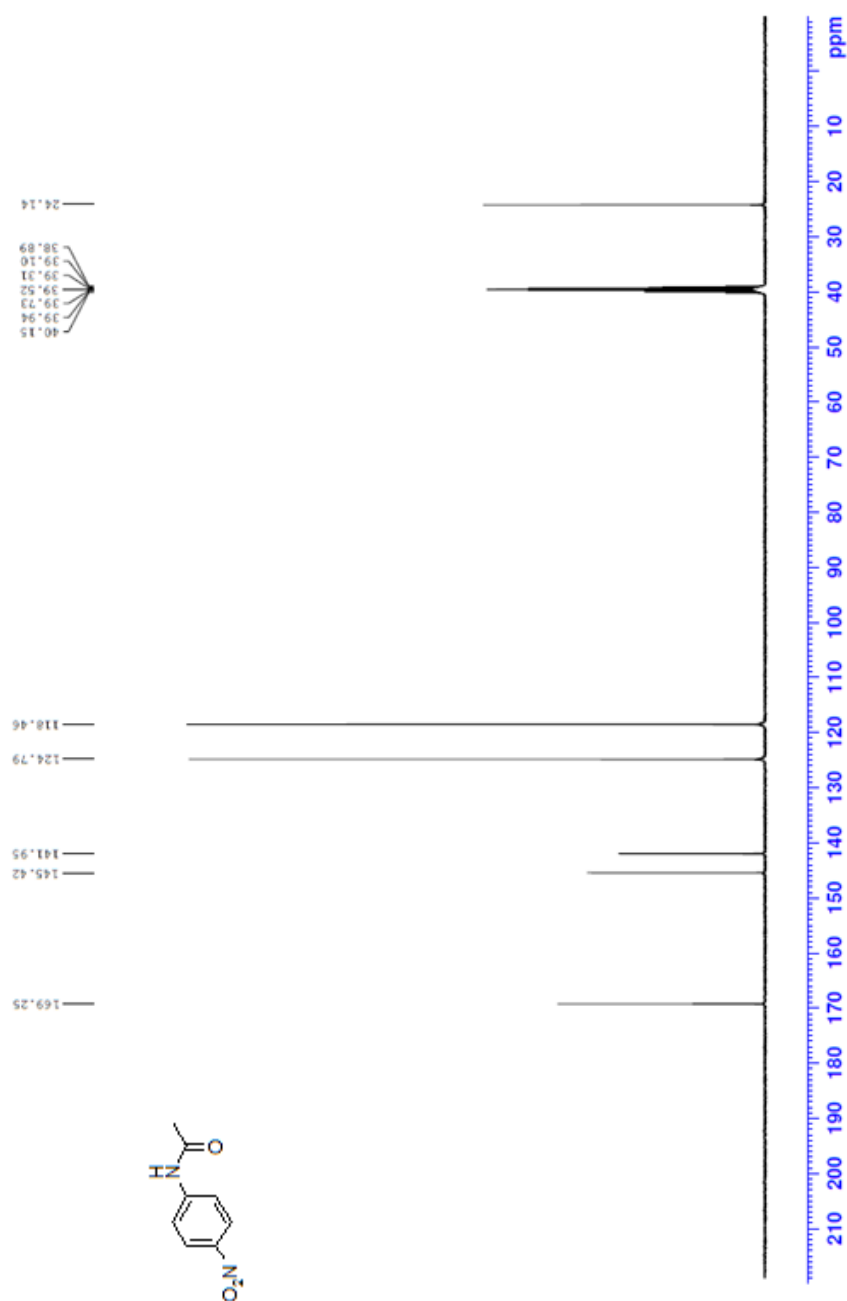




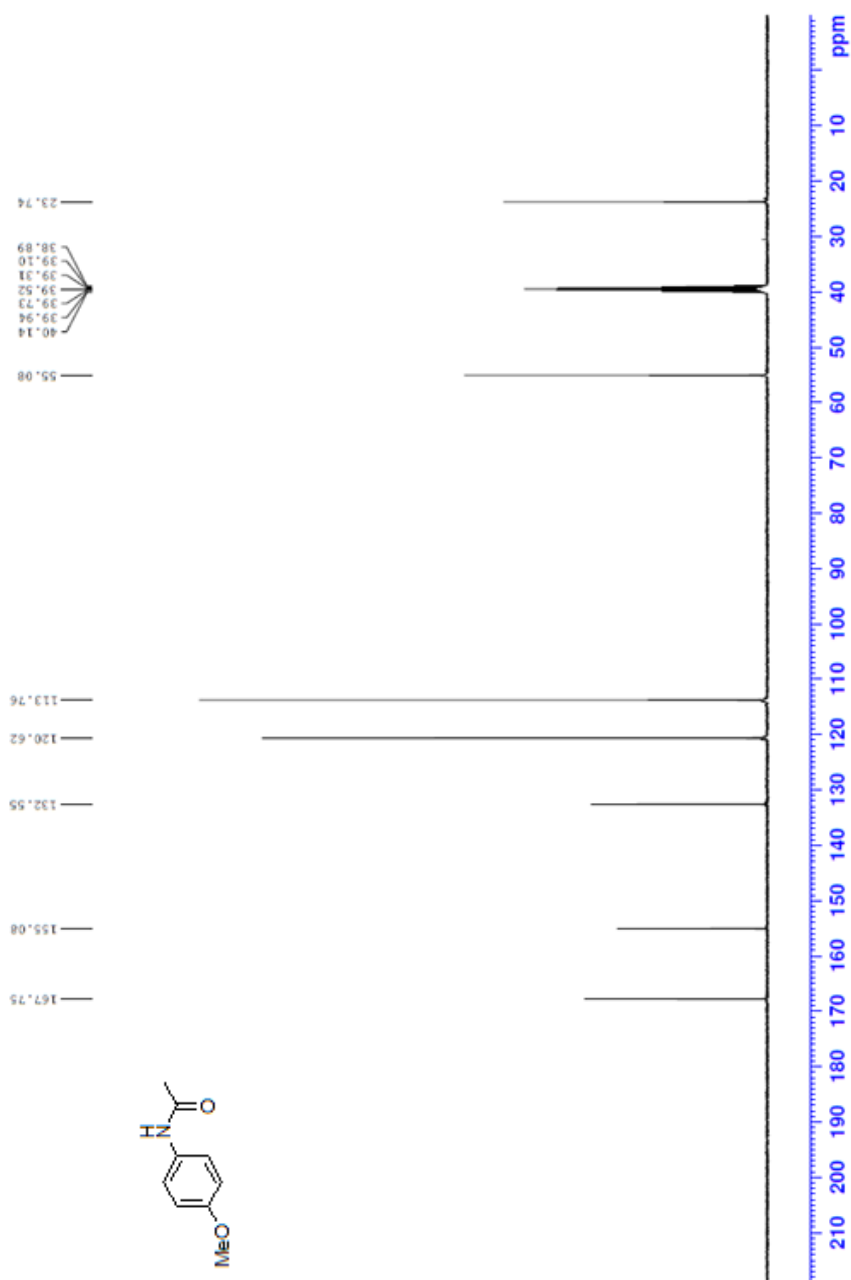




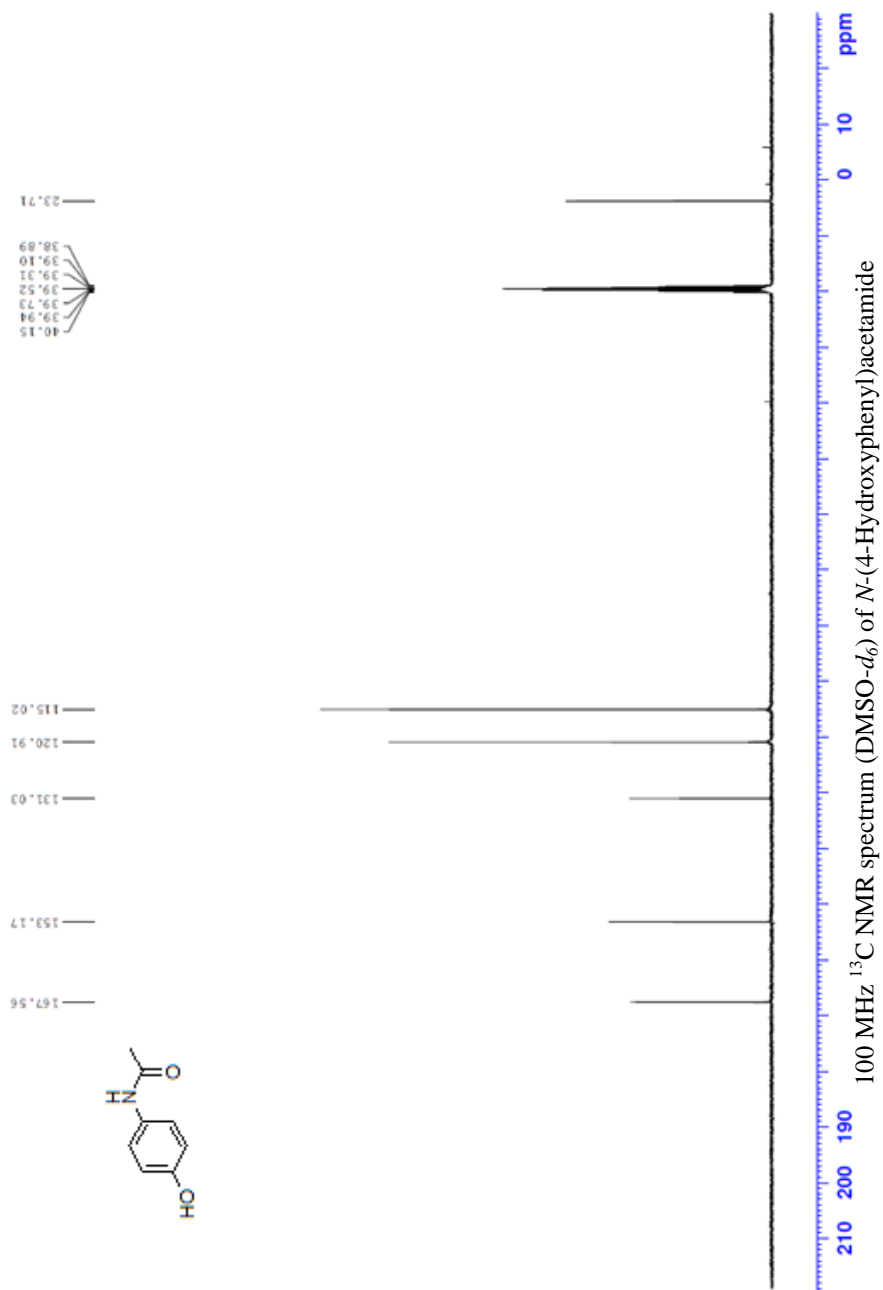




100 MHz ¹³C NMR spectrum (DMSO-*d*₆) of *N*-(4-nitrophenyl)acetamide



100 MHz ¹³C NMR spectrum (DMSO-*d*₆) of *N*-(4-Methoxyphenyl)acetamide



국 문 초 록

학번: 2011-30278

이름: 서 영 란

본 논문에서는 *N*-하이드록시메틸기를 갖는 안정한 α -아미노 알데하이드를 β -아미노- α -하이드록시산을 입체선택적으로 합성하는데에 활용한 합성법을 보고함과 동시에, 본 합성법을 활용하여 *N*-하이드록시메틸기를 갖는 다양한 α -아미노 알데하이드로부터 생리활성을 갖는 물질을 합성한 예들도 보고하고 있다. β -아미노- α -하이드록시산은 높은 입체선택성을 갖으며 합성되는데, 이는 α -아미노 알데하이드와 페닐설폰닐나이트로메테인의 반응을 통한 *N*-하이드록시 메틸기의 분자내 첨가반응을 통해 가능하였고, 합성된 β -아미노- α -하이드록시산의 절대배열은 두 개의 알려진 β -아미노- α -하이드록시산의 합성을 통하여 확인되었다. 정립된 합성법은 아미노펩티데이지의 억제제로 알려진 베스타틴을 합성하는데 적용되었으며, 적용된 성공적인 펩타이드 합성방법을 토대로 아이소부틸과 아이소프로필 결가지를 갖는 베스타틴 유도체를 처음으로 합성하였다. 이와 더불어, 글루타메이트 차단제인 *L*-threo- β -벤질옥시아스팔틱산의 합성도 *N*-하이드록시메틸기의 분자내 반응을 통하여 가능하였다. 마지막으로 지금까지 입체선택성이 밝혀지지 않은 랑스타틴의 입체선택성을 확인하기 위하여 *threo*-3-

아미노-2-하이드록시-4-메틸 펜타노익 산을 포함하는 펩타이드를 처음으로 합성하였다.

본 논문의 후반부에는 아세트아미노펜의 친환경적인 공정에 관하여 논의 되었다. 새로이 제시된 공정은 *para*-다이할로벤젠으로부터 효율적으로 아세트아미노펜을 합성하는 방법이 소개되었고, 소개된 공정을 통하여 기존 아세트아미노펜의 공정이 갖는 문제점이 해결되었으며, 동시에 경제성이 있는 금속촉매 반응을 통하여 친환경적인 공정환경을 조성할 수 있었다. 두 단계 반응을 통하여 *para*-브로모아이오도벤젠은 *N*-아세틸-*para*-아미노페놀로 79%의 수율로 전환되었고, 더 간소화된 단일단계 반응을 통하여 효율성과 경제성을 높임과 동시에 두 단계 공정에서와 유사한 수율(74%)로 아세트아미노펜을 합성할 수 있었다.